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BREAST FEEDING  
AND  
ARTIFICIAL FEEDING

A Clinical, Serological, and  
Biochemical Study in 402 Infants, with a Survey  
of the Literature

*The Norrbotten Study*

OLOF MELLANDER, BO VAHLQUIST,  
TORE MELLBIN  
AND COLLABORATORS

STATISTICIAN: GUNNAR EKLUND

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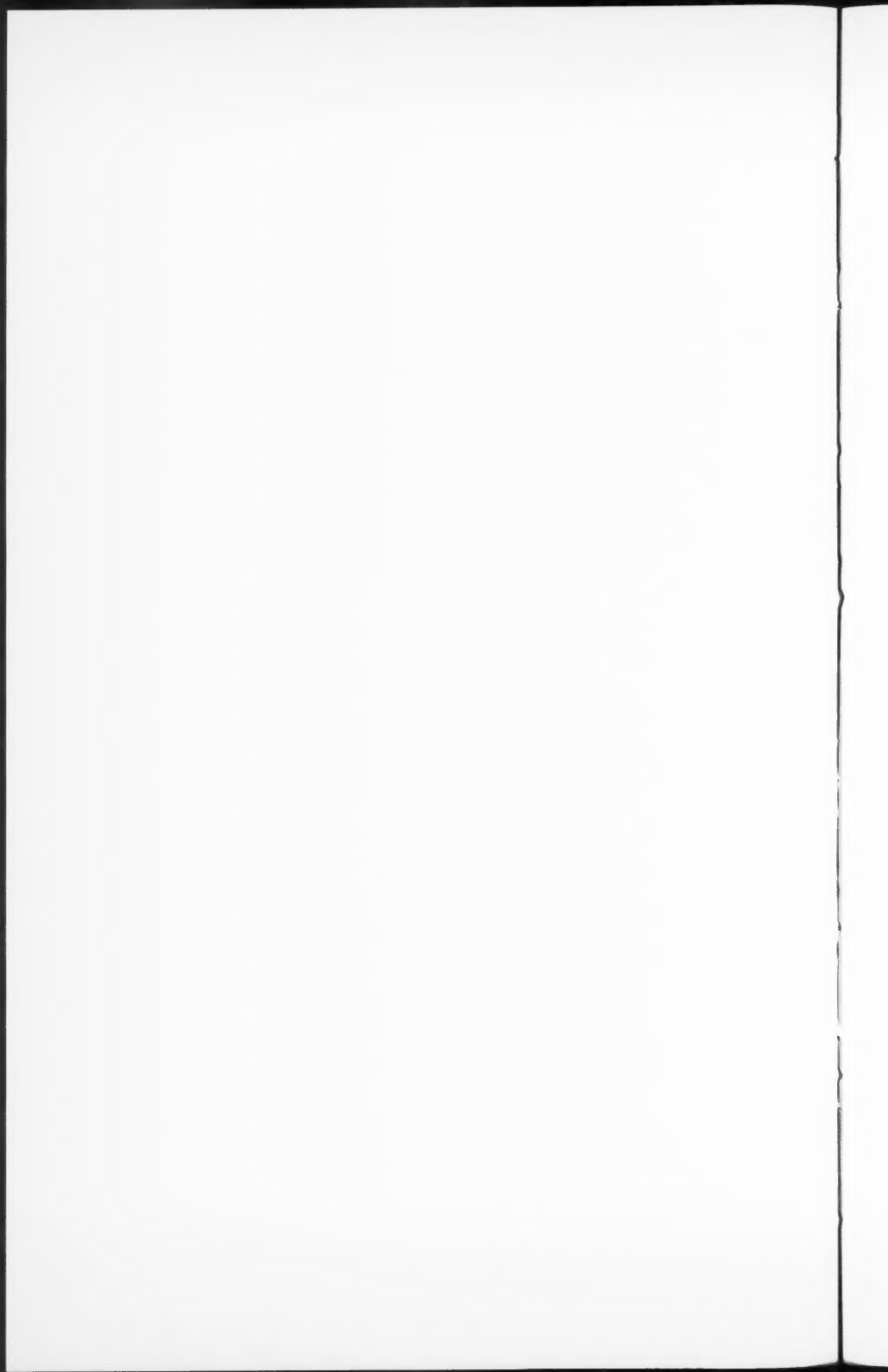
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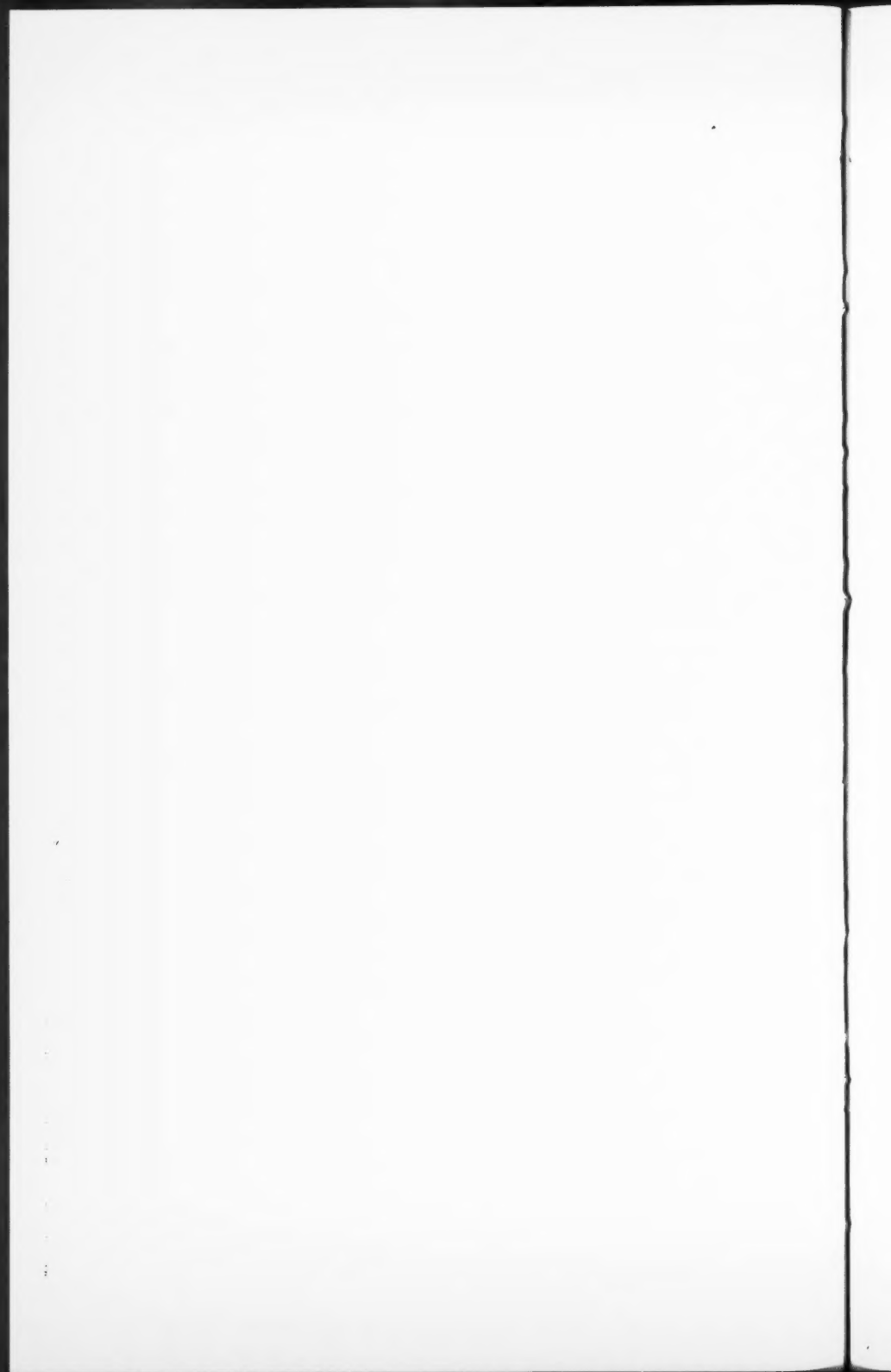


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*This investigation was made possible by the financial aid of the County Council of Norrbotten, Sweden, to whose members the authors extend their sincere thanks for their constant interest and wholehearted support.*



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UPPSALA 1959  
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The contributors are listed in alphabetical order.

## PREFACE

Over the years there has been constant discussion about whether cow's milk and other heterologous milks can provide a satisfactory substitute for the homologous human milk during the early part of the child's life. Common experience has shown that with up-to-date methods of feeding with cow's-milk mixtures, weight-gain and growth are comparable with those of the breast-fed infant, and that the general health is satisfactory. This does not necessarily mean that the bottle-fed baby is in every respect indistinguishable from the breast-fed, however. Of the many comparative studies that have been made, strikingly few have been based on detailed analysis of serological and biochemical data in relation to clinical findings in a series in which the groups to be compared differed solely with regard to mode of feeding, thus conforming with the fundamental requirements of any comparative investigation. In the work now presented these factors were taken into consideration.

The investigation was carried out in Norrbotten, the northernmost county of

Sweden. It could have taken place anywhere in the country, since the incidence of breast feeding is in most districts still sufficiently high. Norrbotten was chosen because preliminary discussion with representatives of the County Council showed that interest in the project was lively and that funds would be made available to carry it through. The considerable problems of administration and of transport of samples in such a widespread and distant area were more than compensated by the support and interest afforded us from the start by the County Council.

After due preparation, the investigation was commenced on June 1st, 1953, and it continued until May, 1957, lasting four years in all. Many people have taken part. We wish to mention two of them in particular, who by their vigorous and whole-hearted support have been indispensable to the carrying out of the investigation: K. G. Viklund, Esq., Chief Administrator of the County Council, and Miss Ebba Nilsson, Head District Nurse.

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## CHAPTER I

# BREAST FEEDING AND ARTIFICIAL FEEDING—A SURVEY OF THE LITERATURE

by PAGET STANFIELD

During the last fifty years continuous and considerable changes have taken place in the knowledge of and attitudes to breast and artificial feeding. In addition, the stimulus, and thus the emphasis and to some extent the outcome of research on the problem has been altering. In this chapter the subject will be reviewed in its development from early comparisons of statistics to the present-day complex comparisons of metabolism.

The first stimulus arose from the necessity to find a suitable alternative should breast feeding fail, an occurrence which fifty years ago imperilled the health and life of the infant in temperate climes, and unfortunately still threatens the millions of infants born into the teeming, unhygienic life of the tropics. Research was rewarded, and its success shown by comparisons of the results of breast and artificial feeding. Vitamins and hygiene, and pasteurization, certification, and homogenization of milk all played a part in this progress.

As women became more emancipated and artificial feeding safer, babies were weaned earlier from the breast. The pendulum commenced its swing in favour of artificial feeding, and provoked demonstrations that breast feeding was still the

best form of nutrition for the baby. This was soon followed by a desire on the part of paediatricians to know whether there was in fact any real difference between the two methods of feeding as regards morbidity and nutrition under the new conditions.

In recent years the controversy between those who maintain the advantages of the breast and those who see no disadvantage in the bottle has been superseded by experimental efforts to clarify the chemical structure and nutritional importance of different milk constituents. In the second place, the desire to find the criteria of optimum nutrition has given new impetus to metabolic-balance investigations, and to more carefully controlled field studies. These latter have involved more exact observation of the details of feeding which were so often omitted or undefined in earlier work. Most authors classify their infants into the wholly breast fed, the partially breast fed, and the wholly artificially fed, but often fail to define their criteria.

In this review an attempt will be made to trace the predominant theories and developments during the past fifty years, under the following headings.

(1) Comparisons between gross statistics

on mortality and morbidity, both general and specific.

(2) Comparisons of development as measured by weight, height, ossification centres, and tooth development.

(3) Comparisons of nutritional status as assessed by blood chemistry.

(4) Comparisons of metabolic studies.

(5) Conclusion. The concept of optimum nutrition.

## 1. Comparison between Gross Statistics on Mortality and Morbidity

The earliest and still the most important approach to the comparison of breast and artificial feeding is that based on mortality and morbidity. Most studies show that mortality and morbidity are higher in the bottle fed. This statement needs further explanation, however.

Figures available over the last 50 years show that the mortality and morbidity during the first year of life have been steadily falling. In the same period the duration and incidence of breast feeding have been decreasing, though at different rates in different countries. There is no doubt that the decrease in mortality and morbidity has been greater in the artificially fed, and so much so that nowadays many people believe that there is no difference between the two methods providing they are both carried out under good conditions.

Böckh, one of the earliest observers, studied the infant mortality in Berlin during 3 one-year periods at intervals of ten years (1885-6, 1895-6, and 1905-6), and related these to the type of feeding. The mortality rate under one year of age was much higher in the artificially fed, but the mortality among the latter fell at a much greater rate than that among the breast fed in the three successive periods.

Though no single observer has since straddled such a period of time, this trend

is clear from comparisons of subsequent studies. In America, Woodbury in 1922 found the monthly mortality figures for 22,000 infants to be 3-6 times higher among the artificially fed. Grulee, Sanford, and Herron (1934) showed mortality figures ten times higher in the artificially fed in Chicago during the period 1924-29. In 1930, however, two studies on small numbers of babies in good condition showed that morbidity could be so reduced in the artificially fed that in fact there was a slightly higher incidence of disease among the breast fed (Glazier, 1930; Faber and Sutton, 1930). In the only subsequent large study on mortality figures in the American continent, Ebbs and Mulligan (1942) from Toronto found equal numbers of deaths amongst 1500 breast- and artificially-fed infants on admission to hospital, though there was still a higher proportion of artificially-fed infants among those admitted, compared with a control group of healthy infants. Stevenson (1947) found among 263 Boston babies only a slight increase in incidence of respiratory infections in the artificially fed as compared with the breast fed. Norval and Kennedy (1949) surveyed 417 infants followed up for at least a year in a well-baby clinic in Rochester. The wholly artificially-fed infants showed an average illness rate during the first year of life of 1.16 per infant, whereas

the rate for breast-fed aged 3 months and over was between 1.50 and 1.57 per infant.

In Britain the same trend is apparent. At the beginning of the century, Howarth (1903) in Derby demonstrated a much higher mortality among the 'hand-fed' babies than in the breast-fed, especially when sweetened condensed milks were used. Smellie (1939) found 41 % of 375 cases of infantile diarrhoea aged under 9 months who had been breast fed for more than 1 month. In these the mortality rate was 29 %, in contrast to 76.6 % among those who had never been breast fed. Robinson (1951) reported her experiences in a poor district of Liverpool during the years 1936-1942. Among over 3000 infants followed up from 3-4 weeks to 7 months or more, there were about equal numbers of totally bottle fed and totally breast fed (about 900 of each). The mortality of the bottle fed was 5-6 times as great and the morbidity twice as great as the breast fed.

Deeny and Murdoch in 1941-1942 compared the feeding of 554 infants dying in the first year of life in Belfast with that in a control group of normal infants selected at random. About 75 % of both groups were breast fed, with or without milk supplement, for the first 6 months of life. The percentage of breast-fed infants alive and well of the total number in each month in the mortality group was lower by just under a half than that of the control group up to the 6th month.

Douglas (1950) compared a sample of 4609 full-term infants born in March, 1946, in Britain, and re-examined when 2 years old. He found that the mortality figures were small, and did not differ significantly

between the artificially fed and the breast fed, though the morbidity rates were slightly higher among the former.

Crosse, Hickmans, Howarth and Aubrey (1954) compared the mortality of breast- and bottle-fed infants in Birmingham. Among 1061 infants weighing  $5\frac{1}{2}$  lbs. or less at birth, the mortality between 1 and 6 months of age varied from 0.8 % in the wholly breast fed to 5.6 % in the wholly bottle fed. The corresponding figures for 2717 mature infants were 0.4 % and 1.4 %. This work was quoted by Asher (1952), who gave no further details.

Asher repeated the type of investigation carried out by Ebbs and Mulligan in Toronto, and attempted to determine the incidence and significance of breast feeding in infants admitted to hospital. She found the incidence of breast feeding significantly lower in cases admitted with infections, particularly gastro-intestinal and upper respiratory tract infections, than in 'clean' cases.

The study by Stewart and Westropp (1953) showed only a slightly greater incidence of respiratory infection in the bottle fed compared with the breast fed in 580 normal infants followed up at Oxford for 1 year. This result has been criticized on account of the possible selection introduced in favour of the bottle fed by excluding infants who had been seriously ill at or before the age of 2 months, but it nevertheless emphasized the small differences which are being found in recent series.

In Scandinavia, most of the work has been fairly recent. It is interesting to note, however, that, despite a very low infant mortality rate in these countries, Mannheim in 1943-1947 found from analysis

of mortality figures in Stockholm that the mortality risk for artificially-fed infants was two to three times greater than for breast-fed. The interpretation of this result is difficult, however. Joensen, in an exhaustive survey of nearly 5000 infants in Denmark during the years 1939-1942, found that overall morbidity rates were higher in the artificially fed than in the breast fed.

Gyllenswärd (1953) compared a group of 142 children followed up in a children's home. Here he had the advantage of being able closely to observe infants in the same surroundings and exposed to approximately the same risk of infection, although on different feeding régimes. He found no difference in the incidence, duration, or severity of clinical infections, and the degree of fever between the breast fed, partially breast fed, and wholly artificially fed.

von Sydow and Faxén (1954) repeated this form of investigation in another children's home on a smaller series of infants. They attempted to exclude all factors which might distinguish the breast-milk-fed and the cow-milk-fed infant by bottle-feeding equal numbers with the two types of milk. Their series was very small: in 48 infants, of whom half were fed with cow's milk and half with human milk, they found the number of febrile periods greater among the former.

The same type of investigation, bottle feeding with breast milk and cow's milk in a carefully controlled environment, is being used in current metabolic studies on full-term infants, and has been employed quite extensively in studies on premature infant feeding (see p. 26). It is hardly amenable to large-scale study of morbidity and mortality, however.

German work in this field has been limited. The early work of Böckh in Berlin has already been described. Since then no major field studies have been reported. Wendtland (1937) studied 327 sick infants of which the majority were treated at a clinic. She noticed the morbidity to be higher in the breast fed than the artificially fed, and ascribed this to malnourished mothers feeding their babies too long on insufficient breast milk, when the bottle-fed infants were receiving more food and care. In such small series, local factors may greatly influence the findings.

Sinios (1955) studied small numbers of infants fed on evaporated milk and pooled breast milk, and found no significant difference in the amount of gastro-intestinal disturbance in each group. This work was criticized with regard to the methods used for comparison, by Droese and Stolley (1955).

The discussion has thus far been limited to the total mortality and morbidity in relation to the predominant type of feeding during the first year of life. Closer observations have been made on, (a) monthly mortality and morbidity figures in relation to the degree of breast or bottle feeding, (b) the long-term effect of breast feeding, and (c) specific morbidity figures.

#### *(a) Monthly Mortality and Morbidity Rates*

In a closer examination of the morbidity rates month by month, most recent studies show that the advantage held by breast-fed infants in the first few months of life decreases, until after three to six months there is no difference in the morbidity rates for breast and bottle feeding

(Joensen, 1954; Crosse *et al.*, 1954; Mannheimer, 1954). If total breast feeding is prolonged to a year, the opposite is reported in those few series in which this has taken place, especially when the nutrition of the mother has also been poor (Orkney, 1946). As artificial feeding has improved, it would appear to have restricted increasingly the advantage of breast feeding to the first three months of life as far as incidence of disease is concerned.

In most of the work already quoted, the authors have divided their feeding groups into (I) totally breast fed over a certain period (usually 3 or 6 months, and often allowing for a certain amount of complementary feeding), (II) wholly artificially fed from 4 weeks onwards, and (III) partially breast fed. They have usually found that the mortality and morbidity figures in this third, mixed group fall conveniently somewhere between the two extremes. A few authors, however, report peculiarities specific to this group, revealed by monthly or tri-monthly examination.

Stewart and Westropp (1953) reported a much higher morbidity risk during the weaning period (from breast to bottle), especially in gastro-intestinal disturbances and especially if weaning occurred late (in the 6th month).

Orkney (1946) found in a district of Calcutta that the partly breast fed had the lowest mortality during the period from 9 to 12 months of life. This was in a community where the mothers' nutrition and also the hygiene of artificial feeding was poor. Her conclusion contrasts strangely with more recent studies by Welbourn (1954) in Uganda, and others in similar, poorly nourished communities, which have shown a marked increase in

the incidence of deficiency and infectious disease during the weaning period. This must be explained by differing local conditions. Finally Mannheimer (1954) in Stockholm found the mortality risk to be lowest among partly breast-fed infants in the second and third trimesters of life. These figures he found impossible to explain.

#### (b) *The Long-term Effect of Breast Feeding*

One or two workers claim to have demonstrated by study of monthly statistics a prolonging of the lowered morbidity of the breast fed after the cessation of breast feeding. Woodbury (1922), in a careful study of monthly mortality statistics in America, stated that monthly death rates were higher among the artificially fed than among the exclusively breast fed, and this difference was proportional to the previous duration of artificial feeding up to the 9th month. This protective influence of breast feeding over the subsequent course of the infant's career has been suggested by other authors, rather in respect of development than morbidity rates. Hoefer and Hardy (1929), analysed the infant-feeding histories of 383 elementary school children from the ages of 7-13, and correlated the subsequent general health and susceptibility to infection to the duration of breast feeding. Except for the prolonged-breast-feeding group (10-20 months), they concluded that the breast-fed infants were healthier, with less infection than the wholly artificially fed. A retrospective study of this kind may be grossly biased and, thus, has been viewed with much scepticism. A similar doubt must be entertained about Douglas's note (1950) that

breast feeding during the early months conferred an immunity to measles that persisted throughout the 2nd year of life. Robinson (1940) stated that the antirachitic effect of breast milk which had been noted in a control group of breast-fed babies (in a comparison with bottle-fed groups) persisted for months after weaning. This was also suggested by Andersen, Rothe-Meyer, and Tudvad (1949).

### (c) *Specific Morbidity Figures*

The general fall in the mortality and morbidity rates, and the decrease in the difference between the rates associated with artificial and breast feeding, are related in two respects. Firstly, increasing hygiene has eliminated the main danger of artificial feeding, that of infection. Secondly, increasing knowledge of nutritional requirements has greatly reduced the incidence of the deficiency diseases which were more frequent in the artificially fed.

Whereas infections and deficiency disease have been decreasing, the incidence of 'allergic' disease has shown a relative increase. Since a large proportion of so-called allergic conditions have been attributed to ingested proteins, including cow's milk, this form of morbidity now merits consideration in any nutritional survey.

In most of the papers dealing with the mortality and morbidity figures in connexion with feeding, the incidence of named disease is also included.

### INFECTIONS

These comprise three main groups, namely, infantile diarrhoea, respiratory infections, and virus infections.

*Infantile diarrhoea.* Until recently this

was the chief offender in the high morbidity and mortality of artificially-fed infants. In studies on infantile diarrhoea, both the incidence and severity of the condition have been considerably higher among the bottle fed than in the breast fed. (Smellie, 1939; Gairdner, 1945; Mayes, 1947; Prince, Gastonia, and Bruce, 1948; Ylppö, Hallman, Donner, Louhivuori, Nevanlinna, and Yliuokainen, 1950).

The same is true of *respiratory infections*. The incidence of these is higher in the artificially fed during the first 6 months of life in all series. Even in most series in which the difference between breast and artificial feeding is minimum, it has nonetheless existed (Stevenson, 1947; Stewart and Westropp, 1953).

*Specific virus infections*, e.g. measles, have also been reported as less frequent in the breast-fed infant (Douglas, 1950; Joensen, 1954), but opinions vary (Atkins, 1958).

Under conditions of unsatisfactory hygiene, with increased opportunity for milk contamination, the higher incidence of these infections, particularly gastro-intestinal, in the artificially fed, have clearly had little to do with the ingredients of the milks themselves. Recently, however, a considerable amount of work has been done to establish whether breast milk exerts a specific protecting influence against infection, and particularly respiratory infection.

*The possible protection afforded by breast milk has been investigated along the following assumptions,*

- (I) that breast milk contains antibodies which can be utilized by the infant,
- (II) that breast milk contains antibacterial and anti-viral properties of

different nature and active particularly against gastro-intestinal infections,

(III) that the structure and proportions of the proteins and amino acids in human milk are best adapted for the synthesis of antibodies by the infant.

1. Human colostrum contains various antibodies, but their concentration is low from the start, and they decline rapidly during the first few days after birth (cf. Nordbring, 1957).

Available data indicate that the capacity of the gastro-intestinal tract of the newborn infant to absorb antibodies is very limited. Vahlquist and Högstedt (1949) found absorption of homologous diphtheria antitoxin given orally to neonates within 24 hours of birth to be usually less than 0.1 %. In older infants the results of antibody administration were almost completely negative. Brown, quoted by Holt (1955), found no absorption at all in a group of infants given diphtheria antitoxin by mouth within 6 hours of birth.

In a recent study by Du Pan, Scheidegger, Wenger, Koechli and Roux (1959) absorption of only minute quantities of tagged gamma globulin was noted after oral administration to 9 infants aged 1 day-2 months.

These results do not conflict with the well-known fact that antigen-antibody reactions may be elicited by oral administration of antigen of protein nature (cf. Ratner and Gruehl, 1935), or with the demonstration, by immunochemical method, of absorption of heterologous proteins (Gruskay and Cooke, 1955), since in either circumstance no proof is available of more than minute absorption of unaltered protein. The recent observation by

Dóbiás, Balló, and Keményvári (1957) of higher levels of staphylococcus- $\delta$ -antitoxin in the serum of infants fed on homologous colostrum and milk is apparently conflicting, but deserves further study.

The newborn child has a gamma-globulin level which is slightly higher than that in the mother's blood, and shows a rich pattern of antibodies, all transferred in utero via the placenta (cf. Vahlquist, 1958). It should be noted, however, that there are both quantitative and qualitative differences between the bloods of mother and child. Immuno-electrophoresis has shown (v. Mural, 1959; Karte, 1959) that the beta-2 A and beta-2 M globulin fractions are virtually absent from cord blood, and the same is true also of some types of antibodies (eg. typhoid O, and iso-agglutinins) which are localized to these fractions, and of properdin. In human colostrum beta-2 A and beta-2 M globulins and many other immune protein fractions are found, and in mature milk beta-2 A globulin is present. It would have been tempting to assume that the supply of these special fractions to the infant is safeguarded by giving homologous milk products, but the data so far available do not support this. The time-sequence for the appearance of the two immuno-electrophoretic fractions is evidently independent of whether or not the child has ever received homologous colostrum and milk, the typical lines beginning to appear in full-term children between the 8th and 12th days of life in both groups (Karte, loc. cit.); and the serum-complement pattern is the same irrespective of the type of feeding (Török and Szemere, (1959)).

To sum up, the situation in man is in striking contrast to that in many animal

species, in which there is massive absorption of antibodies from the dam's colostrum. The human infant obviously depends for its supply of specific antibody during the first period of life completely or almost completely upon placental transfer in utero.

II. Antibacterial properties have been found in milk (Dold, Wizemann and Kleinen, 1937), but these are non-specific to human milk, and are found in cow's milk as well. Sabin (1950) found an antipoliomyelitic factor in human milk, probably not of antibody nature, which was only detected in very few of the specimens of cow's milk examined.

Much work has been done on the observation that breast-fed infants' stools contain an almost pure lactobacillary flora. György (1953) found a growth factor for *L. bifidus* (Penn.) specific to human milk, and Ross and Dawes (1954) concluded from experiments that the low pH of stools of breast-fed infants possibly combined with an increased concentration of formic acid to discourage the growth of *E. coli*, thus preserving a lactobacillary flora. They suggested that human milk contained a factor (possibly identical with György's factor) necessary for the maintenance of an acid pH and a lactobacillary flora.

Killander (1958) has reviewed the recent work on the isolation and identification of 'bifidus factors' found in human breast milk. A number of factors have been found, and one or two synthesized, which, when added to a cow's-milk mixture, can produce a lactobacillary flora in the infant's stools. The significance of this is not known.

III. Human milk may contain proteins and amino acids in the proportions and

structures which are optimum for absorption and subsequent metabolism into specific antibody substances. Studies have been made on the electrophoresis of the serum of breast- and bottle-fed infants, and no differences have been observed (Crosse *et al.*, 1954; Hassan and Gunther, 1958). Immuno-electrophoresis provides a new means of comparing the effect of different types of feeding, but so far no detailed investigation has been reported.

In experiments on twin lambs and kids, Öberg and Mellander (1955) observed that the antibody response following immunization with influenza A was influenced by the type of milk upon which the young animals were fed, and was greater when homologous milk was used than with heterologous. In the case of another type of antigen, diphtheria toxoid, a less striking difference was noted. As far as is known, no similar experiments have previously been carried out in man.

#### DEFICIENCY DISEASE

##### *Vitamin deficiency*

*Rickets.* In temperate zones, where severe malnutrition is rare, the most important deficiency which has been studied clinically in relation to breast and artificial feeding is rickets.

In most comparisons of breast and artificial feeding, each feeding group has been supplemented with vitamins A, C, and D, and no attempt made to assess the relative deficiencies of these factors. In those studies in which an incidence of rickets is reported, the weight of evidence is that the condition is commoner in the artificially fed (Williams and Kastler, 1934; Andersen *et al.*, 1949; Joensen, 1954; Crosse *et al.*, 1954; Bharucha, 1956). Rollin-

son (1940) reported a comparison between human milk and different modifications of cow's milk, and the effectiveness of anti-rachitic treatment in 240 infants. The incidence of rachitic changes in radiological studies of the bones was estimated. The breast-fed infants were found to need less added vitamin D to protect them completely against rickets than the artificially fed. von Sydow (1946) studied the development of rickets in premature infants. Although he found no difference in the radiological evidence in the breast or artificially fed, he discovered in those cases with radiological signs, biochemical changes suggestive of incipient rickets more commonly in the breast fed than the artificially fed. This may show, as he says, that the phosphorus requirements of the premature infant are not satisfied when human milk alone is given, and the calcium requirements are met only if vitamin D is supplied.

*Vitamin A deficiency* in the form of acute infantile keratomalacia has been found in the tropics in both breast and artificially fed infants as a reflection of the general nutritional deficiency of the population.

Concerning *thiamine deficiency*, Jelliffe (1955) writes, 'Infantile beri-beri is the only grave form of malnutrition which occurs commonly and indeed typically in infants receiving mother's milk in adequate amounts, being entirely due to a thiamine-deficient maternal diet during pregnancy and lactation with consequent inadequate neonatal stores and very low vitamin levels in the breast milk.'

*Pyridoxine deficiency* has been noted recently as a cause of convulsions in infancy. Bessey, Adam, and Hansen (1957), in assessing the requirement of pyridoxine,

described the condition as occurring most commonly in infants fed a pyridoxine-deficient, powdered-milk preparation, but they included two breast-fed cases, in one of which the breast milk was found to contain a very low level of pyridoxine.

*Megaloblastic anaemia of infancy* has been reviewed by Zuelzer and Rutzky (1953). It appears to arise from a complex deficiency in which vitamin B<sub>12</sub>, folic acid, citrovorum factor, and vitamin C may all be involved. They concluded that the condition was most commonly found in infants between 6 and 14 months on a more or less limited milk diet, including occasionally breast milk; its occurrence in infants fed exclusively on goat's milk has been recognized for some time.

Breast-fed infants have been shown to have a higher level of *vitamin C* in their serum (Snelling, 1939; Dann, 1942). Sinkko (1937) in Finland found an inverse relationship between the level of vitamin C in the mothers' milk and the morbidity and mortality of infants. The vitamin C content is highest in the summer months, when the mortality and morbidity are lowest. Whether this relationship is causal is not certain.

No studies on the comparison of clinical vitamin C deficiency in the breast fed and artificially fed could be found, though subclinical scurvy has been described in breast-fed infants in India (Bharucha, 1956), and certainly occurs among the artificially fed. The possibility of subclinical deficiency as a factor in susceptibility to infection has been mentioned. The widespread practice of giving infants supplementary vitamin C has made controlled studies of its significance in the two feeding groups very difficult.

### *Mineral deficiency*

*Iron deficiency.* The only mineral which is in short supply in both human and cow's milk is iron. Even when brought up under the best conditions, infants regularly pass through a period with successive diminution and finally almost complete exhaustion of the iron reserves, from the age of 6 months onwards during the first few years of life (cf. Hagberg, 1953; Smith, Rosello, Say, and Yeya, 1955).

Some data indicate (Feuillen, 1954) that iron is utilized more fully from milk than from other nutrients, but others have failed to corroborate these findings (Schultz and Smith, 1958). Since the milk mixtures used to-day often contain considerably more iron than the human milk (see Chapter II), there seems to be no good reason why the bottle-fed baby should be more prone to develop anaemia than the baby fed at the breast.

In babies born at term and reared under good conditions according to modern concepts of nutrition, the iron-deficiency state gives rise to frank anaemia only in rare cases. The appallingly high incidence of iron deficiency anaemia in children in tropical and subtropical countries is due to many factors including gross iron deficiency in the diet over a long period, and often intestinal blood loss owing to infestation with hookworm.

In this connexion it is appropriate to say a few words concerning other clinical forms of grave deficiency. In most temperate countries such maladies have been more or less completely eradicated. In the tropics and subtropics, however, single and multiple deficiencies are still probably the

greatest cause of disease and death during the first year of life. Jelliffe (1955) has reviewed this whole question, which forms a topic of an entirely different magnitude and structure to that which has been considered in this review. The following quotation from his paper concerning the extremely grave deficiency disease, kwashiorkor, may be justified. This condition is a manifestation of protein deficiency which is still widespread in tropical countries. 'In the prevention of kwashiorkor ... prolonged breast feeding through the first two years of life is undoubtedly a measure of the greatest importance and value.' Jelliffe expands this statement a little more in noting Orkney's experience in Calcutta (already quoted), where she found that, though the artificially fed had the highest morbidity and mortality rates, infants still fed on the breast alone after 9 months became 'breast starved', and showed a morbidity and mortality greater than those who were only partially breast fed. He states that, 'during the first six months, breast milk should form the sole or main food of tropical infants, while after this prolonged breast feeding should serve as a vital protein supplement to other foods'.

### 'ALLERGIC' CONDITIONS

No conclusion regarding the importance of cow's milk in the production of these states has been reached. However, the significance which some workers have recently attached to the matter makes it necessary to consider this aspect of morbidity in connexion with the subject under discussion. This uncertainty depends mainly on the absence of any clearly defined immunological abnormality in us-

pected cases of cow's-milk allergy, and the reliance which has thus to be placed for diagnosis upon the clinical observation of the effect of withdrawal of cow's milk on the symptoms, a factor which is so liable to bias. Thus facts are as scarce in this problem as opinions are numerous.

Figures for the frequency of cow's-milk allergy in paediatric practice vary from 0.3% (Collins-Williams, 1956) to 7% and over (Randolph, 1948; Clein, 1954). The importance which is ascribed to cow's milk in the production of allergic conditions in America is reflected in the work of Glaser and Johnstone (1953), who lay emphasis on the total elimination of cow's milk from the diet of all 'allergic' or 'potentially allergic' infants (the latter signifying all infants with a strong family history of 'allergy'). The study upon which their conclusions are based has been rightly subjected to considerable criticism over the method of selection of the control groups (Lowell and Schiller, 1954), and has not, as far as is known, been repeated. In Europe, much less significance has been ascribed to cow's-milk allergy, and the literature consists almost entirely of isolated reports of acute anaphylactic reactions. Reviews have been published by Vendel (1948) and very recently by Stanfield (1959).

In comparisons of the morbidity of infants, breast- or bottle-fed, mention is seldom made of the incidence of 'allergic' disease. Grulee and Sanford (1936), in a large series, declared the incidence of 'eczema' to be seven times higher in the artificially fed than in the breast fed. This has not been the experience of subsequent authors. Edgren (1942) in a series of 295 eczematous infants found no difference in

the incidence of the two feeding methods, and in addition that the time of onset of the eczema was not affected by the type of feeding. Joensen (1954) found no relationship between eczematous skin disease and feeding until the second 6 months of life, when he noticed a declining frequency in the wholly breast fed. This he admitted was probably influenced by other factors as well, particularly the general care of the infant, which may well have influenced Grulee and Sanford's figures also.

Allergic conditions may be more frequent among infants fed on cow's milk, but the comparisons thus far documented either show that this difference is insignificant or are too uncritical to be accepted without further evidence.

### *Conclusion*

The most noteworthy work on the comparison of breast and artificial feeding with respect to mortality and morbidity has been reviewed. The comparison is by no means simple, for there are many factors which may influence both the incidence of breast and artificial feeding and the mortality and morbidity. Social conditions, size of family, position in the family, initial feeding difficulties, congenital abnormalities, and individual constitutional differences are some of the more obvious of these. Furthermore, morbidity itself may affect the incidence of breast feeding; the reaction of some mothers to the mildest indisposition of the infant is to wean him from the breast.

There has been no space to detail the attempts of each investigation to observe or control these factors, either by side inquiries into the incidence of breast feeding and of morbidity in relation to each

of them, or by elimination of as many as possible in the study of much smaller groups in which more controls and fewer variables enter (Jochims, Sager, and Ebel, 1953; von Sydow and Faxén, 1954).

It is concluded that the mortality and morbidity rates in infants reared under less than optimum conditions favour the breast fed. This is largely due to the opportunity for infection transferred to the milk or to the infant during artificial feeding, and,

in the past, to the use of poor-quality, artificial milk mixtures.

Closer scrutiny in recent studies, carried out under the best available conditions, still shows some slight increase in incidence of infection, proneness to deficiency disease, and possibly a greater risk of allergy in the artificially-fed, compared with the breast-fed infant. This difference is in most investigations limited to the first three months of life.

## 2. Comparison of Development as Measured by Weight, Height, Ossification Centres, and Tooth Development

At the same time as gross impairment of health has been studied, increasing interest has been shown in comparisons of the nutritional value of breast milk and cow's milk as measured firstly by weight and height, and more recently by other standards of development.

### *Weight*

One of the first studies on weight gain in the two feeding groups was done by Höjer (1926) in Stockholm. This was a cross-sectional survey of normal infants, about 50 in each month. The graph showed that those reared on cow's milk showed a lower initial gain, but then remained parallel to, but slightly below, the breast fed for 6 months.

Since then, a number of workers have found that breast-fed infants gain more weight during the first 6 months than do the bottle-fed (Hoefer and Hardy, 1929; Williams and Kastler, 1934; Ford, 1949; Gyllenswärd, 1953; Crosse *et al.*, 1954). In more prolonged periods of study to 1

year, some authors have shown that artificially-fed infants overtake the breast-fed infants at about 3 months (Faber and Sutton, 1930), or at about 6 months (Glazier, 1930; Joensen, 1954), to become on an average heavier than the breast fed at the end of one year.

Some workers have found no difference in the weight gains during the first six months (Broman, Dahlberg, and Lichtenstein, 1942; Paiva, 1953; Sinios, 1955; Mannheimer, 1954; Thomson, 1955; Gross and Moses, 1956), though Paiva, following up his small series of breast-fed infants, found that the 50 percentile fell below the 50 percentile of standard weight curves after the fifth month (by which time the vast majority of infants making up the standard weight curves were artificially fed).

Isolated weights have been recorded in larger series at 6 months, 1 year, and 2 years of age. Stewart and Westropp (1933) found the average weight of 580 normal infants to be greater in the artificially fed both at 6 months and 1 year. Douglas

(1950), in 4669 infants followed up at 2 years, found the average weight of the wholly artificially fed to be greater than that of the predominantly breast fed.

At this point mention only will be made of the number of studies comparing the weight curves of premature infants fed on different types of milk during the first few weeks of life. It is clear from these studies that gain in weight is greater in premature infants fed on cow's milk than in those fed on breast milk (Magnusson, 1945; Gordon, Levine, and McNamara, 1947; Rothe-Meyer, 1949; Crosse *et al.*, 1954; Kagan, Hess, Lundeen, Shafer, Parker, and Stigall, 1955). Most of this difference between the groups has been attributed to the greater ash content of the cow's milk, producing a larger obligatory water content in the infant (Kagan *et al.*, 1955). This is only applicable to premature infants during the first month of life, whose kidney function is immature.

### Height

Measurement of height has been less often studied. Hoefer and Hardy (1929) in a retrospective study of 383 Chicago school children mentioned that there was no difference in the subsequent heights of breast- and bottle-fed infants. This was also the experience of Williams and Kastler (1934) in a small series in New Orleans. Broman *et al.* (1942), in a study on child growth in Stockholm, stated that no correlation could be shown between the length of the nursing period and the height and weight after one year. Paiva (1953) found that the 50 percentile for height in his small series of breast-fed infants followed the standard height channel until

the fifth month, after which, with the weight, the height fell away in the 6th month.

### Development of Ossification Centres

Bone development is seldom mentioned in the literature on this subject, except where the incidence of rickets is discussed. Stewart and Westropp (1953) studied the bone development as estimated by the number of ossification centres in the hand, and by vague reference to the size of the fontanelle at 1 year in 580 normal infants. They could discover no relationship between these measurements and the type of feeding.

### Tooth Development

As regards tooth development, observations have been commoner, since no special equipment is required. Durand (1916) referred to the work of Seagrave, who examined the state of the teeth in 2000 children from 2 to 7 years old, and related this to the diet during the first six months of life. There were no differences in dental health or development between children fed on breast milk and cow's milk, though a high incidence of caries was found among those children who had been fed on sweetened evaporated milk. Durand himself confirmed these observations in a study of 600 further cases. Hoefer and Hardy (1929) in a retrospective study of some 400 school children in Chicago stated that dentition had commenced earliest in the group of infants breast fed for 4 to 9 months.

In the same work in which they examined bone development, Stewart and Westropp (1953) counted the number of

teeth present at one year of age, and concluded that this was equal in the two feeding groups. Joensen (1954) found no difference in the number of teeth present in breast- and bottle-fed infants.

In *conclusion*, the weight of the artificially-fed infant is usually initially slightly lower than that of his breast-fed fellow; between the 3rd and 6th month this small difference is obliterated by an increasing

gain in weight by the artificially-fed infant, which results in his being the heavier at one year, and this may still be evident at 2 years.

As regards height, less is known. Gains are about equal, but there is a tendency for the breast-fed infant to gain less than the artificially-fed after the first six months of life. Few data are available concerning the development of osseous centres in different feeding groups.

### 3. Comparison of Nutritional Status as Assessed by Blood Chemistry

(a) *Serum Proteins*. The serum protein levels have been studied mainly with regard to the premature infant in comparisons of breast milk and high protein milk feeding (cow's milk, skimmed citric acid milk, casein hydrolysate-enriched breast milk).

Rothe-Meyer (1949) found a higher level of total serum protein in premature infants fed on protein-enriched breast milk in the first month of life. This correlated with the greater weight gain in these infants already demonstrated by Magnusson (1945) and confirmed by many others.

Crosse *et al.* (1954) could find no significant differences in levels of proteins, both total and separated electrophoretically, in the serum of breast-fed and bottle-fed premature infants during the first few months of life.

Yoshida (1955) studied a group of infants who were breast fed by mothers who showed evidence of clinical malnutrition with lowered serum albumin and raised globulin fractions. He reported that, though the infants showed no clinical manifestations of nutritional dystrophy, the

electrophoretic patterns tended to resemble those occurring in infants with clinical malnutrition. The total serum protein levels remained normal. The work suggests that the composition of breast milk may be affected in conditions of maternal malnutrition.

Hassan and Gunther (1958) measured the total serum protein and the electrophoretic components at 6 months of age in a small series (28) of breast-fed and artificially-fed, full-term infants and found no observable differences.

(b) *Blood urea* levels were studied by Rothe-Meyer (1949). He found that premature infants on breast milk showed lower average levels than those on high-protein milk.

(c) *Blood lipids*. As knowledge on nutrition expands, it is to be expected that other substances will have to be considered both as regards their blood levels and their metabolism in different feeding regimes. Recently an increasing interest has been paid to cholesterol and lipoproteins, particularly in respect to dietary influences on their level in the blood, and the

subsequent development of atherosclerosis. Though extensive work has been done on the blood levels of these substances in infants, including the effect of adding to, or subtracting from, their diet various fatty constituents, few specific investigations into comparison of the levels in the breast and artificially fed have been performed. Pruna (1956) quoted two other Italian workers, Pinna and Marini (1953), as having found a slightly lower mean cholesterol level in the artificially-fed infant, but could demonstrate no significant differences in his own estimation of the cholesterol and fatty acid values in 20 breast-fed and 20 artificially-fed infants aged from one to twelve months. More work in this field is to be expected.

(d) *Vitamin C* levels have been studied in breast- and bottle-fed infants, and found to be higher on the average in the breast-fed (Snelling, 1939; Dann, 1942). This may occur even on equal intakes of vitamin C (Dann). Their figures appear to be significant.

(e) *Serum sodium and potassium*. A number of flame-photometric investigations have been carried out on these metals during recent years, e.g. those of Gyllenswärd and Josephsson (1957). The normal values given by different authors have shown considerable variations, which to some extent can only be explained by analytical difficulties. No classification according to the mode of rearing appears to have been undertaken.

(f) *Serum calcium, phosphorus, and alkaline phosphatase*. There are conflicting reports in the literature on these levels. This is in part due to the fact that the studies have been made on infants already receiving vitamin D. They are therefore

measurements of the effect of vitamin D on breast- or bottle-fed infants, and are dependent also on the faithfulness with which the infant is given and takes his dose.

Williams and Kastler (1934) were the first to mention calcium and phosphorus levels in a series of 60 entirely artificially-fed infants—they did not mention whether vitamin D was given or not. They gave average levels at three and six months.

Gyllenswärd and Josephsson (1957) determined serum calcium at three, six, nine, and twelve months of age. The number of analyses, however, was too small to permit comparison between the groups.

von Sydow (1946) investigated carefully the blood levels of calcium, phosphorus, and alkaline phosphatase in a series of breast-fed and bottle-fed premature infants (below 2,000 g. birth weight) with and without added vitamin D. He compared his results with a series of full-term, healthy, breast-fed infants who were receiving an ample supply of vitamin D (daily dose from October to May of 1000–1500 I.U.). He found that levels of alkaline phosphatase were higher, and those of calcium and phosphorus lower in the breast-fed premature, particularly in those who did not receive vitamin D. The only group of infants who showed values similar to the full-term series consisted of the cow-milk-fed prematures who received an addition of vitamin D. It is doubtful, however, whether blood levels of calcium, phosphorus, and alkaline phosphatase should be assumed to be physiologically similar in the premature, and in the full-term infant given vitamin D.

von Sydow's findings have been recently confirmed by Eek, Gabrielsen, and Halvar-

sen (1957), who studied 69 premature infants all receiving vitamin D from the fourth day of life, and found a tendency towards rachitic blood levels of calcium, phosphorus, and alkaline phosphatase more marked in the breast-milk-fed infants.

(g) *Serum iron and iron-binding protein.* The serum iron and iron-binding protein

show considerable, normal variations during the first year of life (Vahlquist, 1941; Smith, Schulman, and Morgenthau, 1952; Hagberg, 1953). According to the relatively scanty data at hand, there is no difference with regard to serum iron between breast-fed and bottle-fed infants (Möller and Vahlquist, 1946).

#### 4. Comparison of Metabolic Studies

This will be no more than a brief summary of the present position. These studies are mainly in the nature of balance studies, a large number of which have been done on the premature and full-term newborn infant with regard to the various constituents of the diet.

More recently the wider use of isotopes in the labelling of various dietary constituents has opened further possibilities in the investigation of their absorption and metabolism in different feeding regimes. These studies may help to elucidate many of the problems which balance studies both produce and leave unsolved.

Observations made on the premature infant differ somewhat from the full-term, probably on account of the immaturity of the absorptive, metabolic and excretory functions. The following remarks refer to the full-term infant unless the premature is specifically mentioned.

##### *Protein Metabolism*

Nitrogen retention based on the percentage ingested is better in the human-milk-fed infant than in the cow-milk-fed (Barness, Baker, Guilbert, Torres, and György, 1957). However, the amount of nitrogen

retained per unit weight on equicaloric diets is greater in the cow-milk-fed infants (Stearns, 1956). This greater nitrogen retention is not accompanied by any difference in weight-gain, and must mean that the artificially-fed infants have a greater nitrogen-content per kg body-weight. Much earlier Rominger and Meyer (1931) in Germany demonstrated excess storage of nitrogen in infants reared on cow's milk. By administering ad libitum a feed containing 67 cal./100 ml. and 1.7 % of protein Fomon and May (1958) obtained retentions of nitrogen "as large as or greater than those of infants ingesting human milk ad libitum". It is still a matter of dispute what figure can be given for the recommended daily allowance of protein in the case of infants reared on a cow's-milk mixture (Holt, 1959; Gordon and Ganzon, 1959; Hill, 1959).

In a discussion on this topic following a paper by Fomon and May (1957), Gordon quoted his work with Levine that there was certainly no evidence of any difference in relation of water balance to weight gain when comparing premature infants fed human milk with infants fed ordinary cow's milk mixtures. Stearns (1956) has shown that the amount of ena-

tinine in the urine, a measure of muscle bulk, was greater in the cow-milk-fed baby by a small average increment of 2-5 mg %.

It has also been shown that nitrogen-retention is correlated to both the quantity and type of dietary fat, and to the carbohydrate content of the diet.

Holt, Tidwell, Kirk, Cross and Neale (1935) and later Söderhjelm (1952) have demonstrated a higher absorption of fat in the breast-milk-fed infant, which might indicate a higher fat content maintaining the weight increments. Further studies have been proposed on individual protein and amino-acid-nitrogen balances, as suggested by the work of Magnusson in prematures (1945), and the effect of other constituents in the diet on nitrogen balance, studied by Rähä (1956), who found that raising the carbohydrate content of the milk increased the nitrogen retention. This has been confirmed by Cornely, Barness, and György (1957), who found that addition of lactose increased nitrogen retention.

In the premature infant, high protein diets are accompanied by an increase in weight gain as well as increased nitrogen retention. This may be due to a greater protein content of the tissues. Levine and Gordon (1942) and Magnusson (1945) and other observers have found higher urea levels in the blood of these infants, and this has been interpreted as a possible indication that the kidneys are under an increased load. Levine and Gordon, however, found no difference in the water content in the premature on breast or cow's milk. Whether the increase in weight gain has any advantage for the infant is still unknown. Thus far, the most important reason for preferring the high protein diet for the premature, which, of course, can

be based on breast milk (Magnusson, 1945; Rothe-Meyer and Sandøe, 1956), is the opportunity of being able to return the infant to the mother slightly sooner after birth. This increases the chances of successful establishment of breast feeding.

According to Jagenburg (1959) the excretion of *urinary amino acids* are essentially the same both in children fed human and cow's milk. As, however, the nitrogen excretion is greater in children fed cow's milk, the ratio amino-nitrogen to total nitrogen is greater in the urine of infants fed human milk. Furthermore, the excretion of taurine is greater in children fed human milk than in those given cow's milk, whereas the excretion of all other amino acids is not significantly altered when in one and the same child the feed is changed from human to cow's milk.

### *Lipid Metabolism*

Fat balances have been done by a number of investigators. Holt et al. (1935) found a greater absorption of fat in infants fed on human milk than on humanized cow's milk, despite identical iodine numbers of the fat. Ocklitz (1955) and Guilbert, Baker, and Barness (1955), on the other hand, found no difference in the fat absorption in the two groups. In premature infants discrepancies have also occurred. Gordon and McNamara (1941) found poor absorption of fat in premature infants, and suggested that this might be a factor in the poorer weight-gain of premature infants fed isocaloric quantities of breast milk with relatively high fat and low protein content than of partially skimmed cow's milk with the reverse. Söderhjelm (1952), on the other hand, demonstrated a more effi-

cient absorption of fat in the breast-fed premature, even with higher fat content, than in the cow-milk-fed. These were careful observations, and an explanation for the discrepancies has not yet been found. Breast milk itself certainly varies considerably in the content of fat (Hyttén, 1955) and also in the degree of saturation of the fat (Holt, 1955). (Cf. Chapter II).

Hansen (1958) has demonstrated that dietary fat deficiency causes a marked increase in the calorie consumption per unit body-weight. Addition of linoleic acid to such a diet reduced the calorie requirements per unit weight. This should therefore explain why breast-fed infants can maintain satisfactory growth on the lower calorie intake than that of bottle-fed babies.

### *Carbohydrate Metabolism*

Reference has already been made to Râihä (1956) who observed increased nitrogen retention and increased water intake in infants on a high carbohydrate diet. Natelson, Kramer, and Sherman (1948) described a more effective response of blood sugar to lactose in human milk than in cow's milk.

### *Calcium and Phosphorus Metabolism*

The marked difference in content of these two elements in human and cow's milk (see later) have naturally emphasized the problem of comparison of their metabolism in the two feeding groups. Conflicting reports regarding the incidence of rickets, and the differences in blood levels have already been touched upon. This has been partly due to the complications introduced when various doses of vitamin

D have been used, partly to the differing notions of what constitutes the radiological picture of rickets, osteoporosis, and changes in the growing ends of the bone, and partly to what have been regarded as normal levels of calcium, phosphorus, and alkaline phosphatase in the blood of the full-term and premature infant.

Thus, to some investigators the cow-milk-fed baby has been regarded as supermineralized (Rominger and Meyer, 1931; Gardner, MacLachlan, Pick, Terry, and Butler, 1950; Gittleman and Pineus, 1951). They have shown a tendency to hyperphosphataemia and hypocalcaemia in the cow-milk-fed baby; and Gardner *et al.* reported a consistent increase in the size of the parathyroids in these infants, which he related to the high phosphate intake.

The majority of reports, however, veer towards the theory that the breast-fed infant, particularly the premature, receives amounts of calcium and phosphorus which are sometimes insufficient for his needs. This is suggested not by an increased incidence of rickets, or by a decreased rate of growth, neither of which is found in the breast fed: the idea has rather developed from the study of blood levels, as has already been briefly mentioned (von Sydow, 1946), and of balance tests (Stearns, 1939; Benjamin, Gordon and Marples, 1943; and others, discussed by Jeans, 1950). The degree of utilization of calcium in cow's milk was found to be greater than that in human milk, despite the lower intake in human milk. By estimating the relative calcium content of the foetus and infant, Stearns showed that the breast-fed infant, particularly the premature, was not able to maintain his birth

percentage of calcium. Despite this, she found the cow-milk-fed baby 'not clinically as good a baby as the breast-fed infant' (Stearns, 1956).

The surface of the problem, thus skimmed, shows that much more must be learned of the normal physiology of the

metabolism of calcium and phosphorus. Though there seems to be such a difference in the amounts of these two substances ingested and retained in the breast-fed and artificially-fed infant, no certain clinical differences are evident in respect of growth and well-being in spite of this.

## 5. Conclusion. The Concept of Optimum Nutrition

In comparing two modes of feeding it is of advantage if behaviour under optimum nutrition is known. In early investigations, observers were content to compare infants fed on the two regimes directly. This was felt to be satisfactory, for, in most studies, the breast-fed baby fared better in all standards measured, and was taken as being the best nourished. Soon it became clear that the development of a breast-fed baby did not necessarily move along the maximum-line in many standards. Reports were received that the artificially-fed baby gained weight more rapidly, cut more teeth, and possibly passed its milestones earlier than the breast-fed infant. This impression tended to be confirmed by metabolic balance studies, which showed that nitrogen and calcium were retained to a greater extent in the bottle-fed baby. Furthermore, if the increase of intrauterine levels of total body constituents was projected into extrauterine life, the course of the artificially-fed infant was found to approximate this projection more closely than the breast-fed, particularly with regard to the premature.

These observations made it necessary to attempt to define what constituted optimum nutrition. It cannot be assumed that progression along, or most closely

approaching, the projected intrauterine line of growth and development is necessarily the best standard for post-natal life. Periods of rest and activity, storage and utilization, are recognized and physiological. Nor can it be assumed that the maximum rate of development in any measurement is necessarily the most favourable; this view would take no thought for the ultimate outcome. Furthermore, the development of the breast-fed infant can no longer be taken as being the most satisfactory, for the amount and also the quality of breast milk is affected by the mother's nutrition and general condition, especially during the later months of breast feeding.

The well-considered comments of Platt and Moncrieff (1947) are still valid to-day. Optimum nutrition cannot be judged by absence of clinically recognizable signs of malnutrition or necessarily by maximum rate of growth. The slow impairment of health, and the health and well-being into adult life must be observed and measured, often only by means of special investigations. 'The signs of optimal progress in development towards maturity may again be difficult to establish, both backwardness and precociousness being sub-optimal.'

Criteria for such 'optimal growth, development, and performance at maturity' with which the nutritional and other merits of human and cow's milk can be compared have not yet been established, and follow-up into adult life of the breast-fed and artificially-fed infant has not been

undertaken. The purpose of this review has been to try to indicate the present position and the lines along which new facts are emerging which will necessitate continual reconsideration of old assumptions.

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## CHAPTER II

# COMPARATIVE BIOCHEMISTRY OF HUMAN AND COW'S MILK

by OLOF MELLANDER and BO VAHLQUIST

The following account is not meant to present a full picture of the subject, but only to mark some of the recent advances.

There is no food that in its composition is so closely adjusted to the requirements of any specific age-group as milk. Different species of animals show great differences in the rate of growth, and this is reflected in the composition of their milk. From a clinical point of view, it is the differences between human and cow's milk that are of the greatest interest. Whereas in the past only very rough data have been available, we are now able to make more delicate comparisons of the two types of milk, since many formerly unknown and often highly important constituents have become demonstrable and accessible to quantitative estimation.

Nonetheless, analytical techniques are

still in many respects unsatisfactory, for instance, with regard to free peptides and certain carbohydrate components, and there is every reason to suppose that further, yet unknown constituents will later be defined.

## PROTEINS

The total protein content of the *mature milk* of any species is related to the rate of growth of the young of that species, slow growth being matched by low protein content. The slowest rate of growth and the lowest milk protein content are encountered in man. The casein content of breast milk is only about  $\frac{1}{6}$  of that of cow's milk, but the whey-protein content, both relative and absolute, is higher. Table 1 is based on our own values from samples of pooled milk.

TABLE 1. *Protein composition of human and cow's milk. The percentage figures given are based on data from samples of pooled milk.*

	Human milk	Cow's milk
Total protein	1.5	3.5
Casein	c. 0.5	3.0
Total whey protein	c. 1.0	0.5
Serum albumin	traces	traces
$\beta$ -lactoglobulin	not yet demonstrated	c. 0.2
$\alpha$ -lactalbumin	amount unknown	c. 0.1
Slowly-migrating components (including immunoglobulin)	easily demonstrable amounts	insignificant amounts

TABLE 2. *Comparison of elemental composition of human and bovine casein*

	N %	P %	S %	N/P	N/S
Human	14.43	0.44	0.73 <sup>1</sup>	32.79	19.77
Bovine	15.24	0.83	0.79	18.36	19.29

<sup>1</sup> The sulphur content of human casein varies according to the method of preparation. (Mellander, 1947.)

*Caseins.* From a physico-chemical point of view casein may be said to constitute a complex protein system, no matter what its origin. On electrophoresis three components are distinguished (Mellander, 1947), and still more can be detected by other techniques. In certain respects clear-cut differences exist. Cow's-milk casein is easily separated from the whey proteins by iso-electric precipitation, but breast-milk casein forms a stable solution at iso-electric reaction. Owing to the difficulty of isolation, breast-milk casein has been subjected to considerably less comprehensive investigation than cow's-milk casein. As can be seen from Table 2, the two types of casein differ in some respects, particularly in regard to the phosphorus content.

Previously it was doubted that notable differences between caseins of different species could occur, partly because distinct antigenic differences had not been demonstrated between cow's-milk and human-milk casein. The observations mentioned above show distinct differences, however, and these become still more apparent when the problem is approached from the biological aspect. The rate of enzymatic hydrolysis of cow's-milk casein is considerably greater than that of breast-milk casein. This applies to both human and bovine enzymes. (Mellander, 1947).

The only casein peptides that have been

examined in any detail are the serin-containing phosphorylated peptides that can be recovered after enzymatic or acid hydrolysis. A relatively greater part of the molecule of breast-milk casein seems to be recoverable in the form of such peptides than of that of cow's-milk casein. The elemental and amino-acid composition of these peptides is rather similar. Their most striking properties are their resistance to continued enzymatic hydrolysis, and their ability to combine with metals such as calcium, iron, etc. For further information on this subject and additional references see Mellander (1947, 1950), Österberg (1957, 1959), Bennich, Johansson, and Österberg (1959), etc.

The possibility of competition between different metals for the peptide-carrier molecule should particularly be borne in mind in the light of the excessive intake of calcium and other metals that occurs during cow's-milk feeding. Significant metals such as iron, copper, cobalt, etc. that are present only in small quantities may be thus deprived of the possibility of combining with a protein-residue.

As can be seen from Table 3 (Johansson and Svennerholm, 1956), considerable quantities of carbohydrate can be demonstrated in casein prepared in the usual manner. The difference in sialic-acid content is of special interest in this connexion.

TABLE 3. *Carbohydrate content of casein*

	Hexosamine	Hexose	Sialic acid	Total
Human	1.32	1.98	0.76	4.03
Bovine	0.18	0.24	0.39	0.80

The  *whey proteins*  must still be regarded as largely uninvestigated, at any rate as far as human milk is concerned. It can be seen from Table 1 that at least two of the components isolated from whey are present in only one of the types of milk.  $\beta$ -lactoglobulin, which is present in fairly large quantities in cow's-milk whey, and which can be obtained in crystalline form, has not so far been demonstrated in human-milk whey. An iron-containing, red-coloured protein has been isolated from human milk after addition of iron. Cow's milk seems to contain little or none of this substance (Johansson, 1958, and personal communication). Although both these proteins have been thoroughly investigated chemically, and have been obtained in fairly pure form, nothing at all is known of their biological function. Both seem to be fully equipped with all the essential amino acids. They probably perform specific functions, for example, in connexion with transportation of iron in the case of the red protein.

Similarly the slowly migrating components of the whey are of great interest, since they undoubtedly contain immune globulins. Whereas these components are present in large quantities in both human and bovine colostrum, of the mature products only human milk contains significant quantities of such slowly migrating fractions.

It is highly interesting that proteins seem to be present in both human and bovine whey that are identical with pro-

teins in serum. An albumin fraction identical to serum albumin has been demonstrated by immunological technique in both cow's milk (Peskett, 1932) and breast milk (Hansson and Johansson, 1959, Hansson 1959). The latter authors also demonstrated the presence of  $\gamma$ -globulin in human milk by means of an immunological technique.

The protein content of *colostrum* is very much higher than that of mature milk, and may be as much as 17% in bovine and 15% in human colostrum. In both cases these high values are due to whey protein. A high content of immune globulins is found in both human and bovine colostrum; and in both an effective trypsin inhibitor is present, though in lower concentrations in human colostrum.

#### CARBOHYDRATES

It is significant that galactose and glucose, which are the break-down products of lactose, are absorbed much more quickly than other sugars (Table 4).

TABLE 4. *The rates of absorption of certain monosaccharides, relative to that of glucose, per cent.*

D-galactose	110	D-mannose	19
D-glucose	100	D-xylose	15
D-fructose	43	D-arabinose	9

Human milk contains much more lactose than does cow's milk (7% and 4.8%, respectively). In addition, human milk contains considerable quantities of nitrogen-containing oligosaccharides (0.4%); cow's milk contains no more than about 1/100 of this amount. These last-named

sugars have acquired interest owing to the bifidus-promoting activity some of them have been found to possess (György, 1953). This applies only to a particular type of micro-organism, however (*L. bif. Penn.*), and it is still uncertain to what extent they influence the development of the normal intestinal flora of the infant. Owing to the property of *Lactobacillus bifidus* of breaking down lactose into acetic acid and lactic acid, it is responsible for the acid reaction of the intestinal contents of breast-fed infants. This may affect the absorption of metals such as calcium (the complex-forming peptides above-mentioned are stable only in acid medium, which suppresses the activity of alkaline phosphatase), and may also interfere with the growth of many enteropathic organisms.

#### LIPIDS

The total lipid content of the milk varies considerably from one individual

to another, and even more from one phase of nursing to another in the same mother.

The composition of milk fat depends on the diet of the mother. This seems to be particularly true of human milk, and drastic changes in it can be brought about by altering the diet (Insull, Hirsch, James, and Ahrens, 1958). Under ordinary conditions, with moderate variations in the diet, the fatty-acid pattern of the milk remains fairly constant.

The milk fat of both species consists largely of triglycerides. Small amounts of cholesterol, phospho-lipids, and free fatty acids are found in both types of milk, but the fatty-acid composition of the lipids differs greatly between them. In particular there should be noted the difference in content of poly-unsaturated fatty acids, including the so-called essential fatty acids: this is low in cow's-milk fat, but more considerable in that of breast milk (see Table 5). The nutritional signi-

TABLE 5. *Comparison of various organic, and inorganic components of human and cow's milk.*

Constituent (Values per 100 ml whole milk)	Human milk	Cow's milk	Constituent (Values per 100 ml whole milk)	Human milk	Cow's milk	Constituent (Values per 100 ml whole milk)	Human milk	Cow's milk
Water	g 88	87	Vitamin A	mg 0.06	0.04	Sodium	mg 15	58
Proteins	g 1.5	3.5	Vitamin B			Potassium	mg 55	138
Carbohydrate	g 7.0	4.8	Thiamine	mg 0.016	0.042	Calcium	mg 33	125
Total lipids	g 3.8	3.7	Riboflavin	mg 0.043	0.157	Magnesium	mg 4	13
'Essential fatty acids'	g 0.35	0.10	Niacin	mg 0.172	0.085	Iron	mg 0.05- 0.06	0.04
Ash	g 0.21	0.72	Pyridoxine	mg 0.011	0.048	Copper	mg 0.04	0.03
			group					
			Pantothenic acid	mg 0.196	0.350	Chlorine	mg 43	103
			Folic acid					
			group	mg 0.0002	0.0002	Phosphorus	mg 15	39
			Choline	mg 0.009	0.0013	Iodine	mg 0.007	0.021
			Biotin	mg 0.0004	0.0035			
			Cobalamin	mg trace	0.0006			
			Vitamin C	mg 4.3	1.8			
			Vitamin D	I.U. 0.4-10.0	0.3-4.0			
			Vitamin E	mg 0.6	0.1			

ificance of these fatty acids is still being intensively studied, and the effect of the difference between the two types of milk upon the young infant cannot yet be assessed. In contrast to breast-milk fat, the cow's-milk fat contains more short-chained fatty acids, which accounts for the characteristic smell of butter, but probably has no biological significance.

#### VITAMINS

Milk is an important source of vitamins. Its content of these substances is strongly influenced by the diet, however, and it is therefore difficult to give any norm. Furthermore, in the case of cow's milk, the concentration of certain vitamins can become greatly changed during preparation. Bearing these reservations in mind, a comparison may be made of the vitamin concentrations of fresh human and cow's milk (see Table 5).

#### MINERAL SUBSTANCES

One of the most striking differences between human and cow's milk concerns the mineral composition. As with the protein content, this may be assumed to be fundamentally bound up with the rate of growth of the young animal for which the milk is intended.

In one respect, namely the iron content, human and cow's milk are fairly similar. The difference reported by earlier investigators, who as a rule found a higher iron content in human milk, has not been confirmed in recent studies. Feuillen and Plumier (1952) give the iron concentration of human milk as 0.05–0.06 mg per 100 ml, and of cow's milk collected direct from the udder as 0.04 mg per 100 ml. These

figures tally with those reported by Wallgren (1932) 20 years earlier. The iron-content of commercial cow's-milk mixtures is commonly many times greater, and the artificially-reared infant may receive through the milk 3–4 times as much iron as the breast-fed (cf. Feuillen and Plumier, 1952; Schäfer, 1953).

The mean values for certain mineral constituents of human and cow's milk are given in Table 5. The mineral content of milk is influenced by the diet of the mother or dam, though not so markedly as is the concentration of vitamins.

Above, a comparison has been made between the compositions of breast milk and cow's milk in natural state. Of greater biological significance, of course, would be a comparison of the total consumption of the individual constituents over long periods of feeding with breast milk alone and with a suitable cow's-milk mixture. Table 6 shows some data of interest in this connexion.

TABLE 6. *Comparison between total amount of various nutrients supplied by human and cow's milk.*

	Breast feeding (135 litres of breast milk during 6 months) g	Cow's-milk feed- ing (160 litres of 1:1 milk-and- water-type mix- ture during 6 months) g
Calcium	45.9	89.6
Phosphorus		
Total-P	18.9	73.6
Casein-P	2.2	19.2
Total protein	1,620	2,720
Casein	540	2,400
Whey protein	1,080	320

## CHAPTER III

### AIM OF THE INVESTIGATION

There are many important qualitative and quantitative differences in chemical composition between human and cow's milk. Presumptively, these differences might be taken to influence the nutritional status of the young infant, for whom milk constitutes the predominant feature of the diet and in whom growth is exceedingly rapid. A detailed account of opinions concerning these problems as they have changed through the years is given in Chapter I. In recent times it has been oftener and oftener claimed that the chemical differences between the two types of milk need not in themselves lead to major differences in the results of rearing. This opinion is largely based on purely clinical findings or simple laboratory tests. In the present work particular care has been taken to match careful clinical observations with certain serological and blood-chemical investigations in order to obtain a broader view of the problem.

One of the differences between breast-fed and bottle-fed infants which has attracted much attention is that concerning resistance to infection. Even in recent, carefully controlled studies, an increased susceptibility to infection has in most cases been noted among infants weaned early from the breast. The mechanism behind this difference is still not understood, and there are several possible explanations. One possibility would be a distinct difference in the capacity for anti-

body formation. Certain animal investigations previously cited have shown that this process is influenced not only by gross protein restriction, but also by such differences in diet as exist on feeding with homologous or heterologous milk (Öberg and Mellander, 1955).

The main object of the investigation has been to establish, on as uniform a series as possible, the incidence of infection in different feeding groups, and at the same time to obtain, by means of quantitative studies on antibody formation and serum protein fractions, a basis for the assessment of any demonstrable differences in this incidence of infection.

The clinical side of the investigation has not been limited solely to recording of the incidence of infection. The opportunity was also taken of making a broad assessment of the children's health and development. Particular attention was paid to the skeletal development, which may be particularly readily influenced by the considerable differences in intake of mineral substances and proteins on feeding with breast and cow's milk.

Laboratory investigations included in addition to determination of serum proteins, a series of other blood-chemical estimations to the extent that material was available and that these were considered of interest in a comparative study of this nature.

## CHAPTER IV

# GENERAL BACKGROUND AND ORGANIZATION

by OLOF MELLANDER, TORE MELLBIN, and BO VAHLQUIST

### 1. The County of Norrbotten

The county of Norrbotten (See the map, Fig. 1.) extends from latitude  $65^{\circ}4'$  to  $69^{\circ}4'$ . It has an area of 105,876 sq.km (40,742 sq.miles) and a population of about 250,000, corresponding to an average of 2.4 inhabitants per sq.km (6.1 per sq.mile). The district consists largely of high, mountainous country and forests. The population is concentrated to the valleys and coastal districts. There are 5 towns, of which Luleå, with 27,000 inhabitants, is the largest.

The population is predominantly Swedish, with Finnish and Lapp minorities (ca. 40,000 and 7,000 respectively). These minorities are now largely Swedish-speaking. Large families are still common. The birth-rate in 1954 was 18.6 per 1000 of the total population of the county, and the infant death-rate in the same year was 20.9 per 1000 live births.

The climate is very hard. Temperatures of  $-30^{\circ}$  to  $-40^{\circ}\text{C}$  ( $-22^{\circ}$  to  $-40^{\circ}\text{F}$ ) are not uncommon during the winter, and during summer the temperature may rise to  $+25^{\circ}$  to  $+30^{\circ}\text{C}$  ( $+77^{\circ}$  to  $+86^{\circ}\text{F}$ ). The ground is frozen and, as a rule, snow-covered from October to May. The temperature and hours of daylight in this area are shown in Fig. 2.

There are well-equipped hospitals at 7



Fig. 1. Map of Sweden. Norrbotten county indicated by densely hatched area.

places (Boden, Luleå, Piteå, Gällivare, Haparanda, Kalix, and Kiruna), and there are in addition 7 smaller 'cottage' hospitals. Medical care and prophylaxis are organized on the same basis as in other parts of Sweden (Ström and Johansson, 1950). In 1954 the number of doctors per

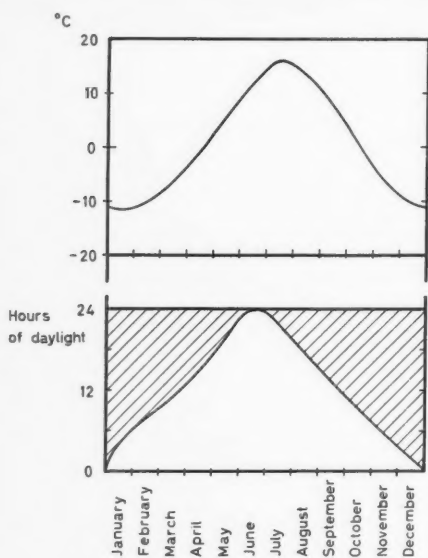


Fig. 2. Mean temperature (above) and hours of daylight (below) at latitude 67°.

10,000 inhabitants was 4.2, and the number of nurses 15.2. The county is divided into 37 provincial medical districts, each with a 'province' doctor who is responsible for the medical care and public health duties of his own district; the purely clinical aspect of his practice takes the greater part of his time. For years there have been difficulties in filling some of these posts. In the country districts it may be difficult at times to maintain contact between doctor and patients, as roads may be blocked by snow.

While the investigation was in progress there were no serious epidemics. There were, of course, outbreaks of morbilli, varicellae, pertussis, etc., but none of diphtheria or severe alimentary infections. The incidence of tuberculosis was formerly high in this part of the country, but here too, during recent years, it has been brought largely under control.

## 2. The Research Regions

Two regions were investigated, Töre-Råneå, a country district, and part of the town of Kiruna. The location of these areas can be seen from the map (Fig. 3). In 1954 the population of Töre-Råneå was 13,192, that of the Kiruna district 12,850. In both areas the population is predominantly Swedish, but in Kiruna there is a small Finnish minority.

Most of the inhabitants of Töre-Råneå are small-holders, with general income-levels lower than in Kiruna, and often with little ready money. The housing situation among most of the population is not entirely satisfactory. The majority of the inhabitants of Kiruna are workers in the big iron-ore mine that gave rise to the

township about 50 years ago. Incomes are on the whole satisfactory, older dwellings are rapidly being replaced by new, and the housing situation is for the greater part of this community satisfactory.

The diet is characterized by a high intake of calories, and relatively high consumption of milk, bread, and potatoes. The consumption of animal fat and protein may be said to be ordinary. Less fruit and vegetables in particular are consumed than in more southerly parts of the country, but typical vitamin-deficiency states are exceedingly rare. Elaborate arrangements exist for the free distribution of supplementary vitamins to pregnant women and young children, through pa-

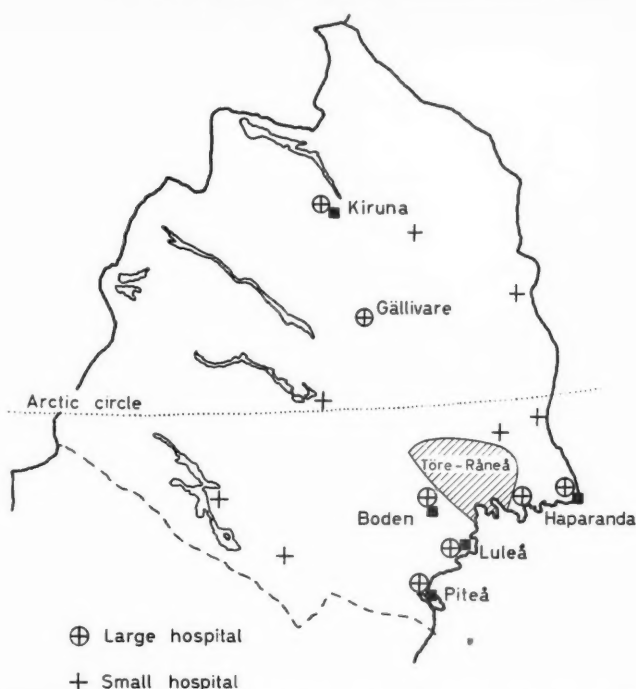


Fig. 3. Norrbotten county with the two research areas, the towns, and the hospitals indicated.

ternity and child-welfare clinics. No clear-cut signs of vitamin deficiency, in either mothers or children, were observed during the whole of this investigation.

During many winter months the cold is so severe that infants and small children can only remain out of doors for very short periods; at times during the summer the gnat-nuisance may have the same effect.

Much superstition and credulity from earlier times have qualified child-rearing in these districts, as in many other parts of the country. During recent years, however, the work of the child-welfare organization has been received with confidence, and the willingness to follow its advice and instructions has been as great as elsewhere.

### 3. Medical Staff

The investigation was conducted from Uppsala and Gothenburg, in which places the laboratory tests were carried out. Upp-

sala is situated about 930 and 1260 kilometres (558 and 756 miles) from Töre-Råneå and Kiruna, respectively. Local

centres were set up at the Paediatric Department, Boden (Dr. Herbert Enell), the Paediatric Department, Gällivare (Dr. Erik Sahlin), and Kiruna Hospital (Dr. Erik Eriksson).

In both of the districts we were assisted by the existing medical and nursing staff, but the staff of the child-welfare clinics was augmented as follows. In Töre-Råneå an extra, full-time nurse was appointed for the entire period of the investigation, and for most of the time also an extra district nurse. The child-welfare organiza-

tion in Kiruna was at the start of the investigation working under considerable difficulties. An extra medical officer was appointed at Gällivare Hospital with part-time duties at the child-welfare clinic in Kiruna, 120 kilometres (72 miles) away. This doctor spent two days per week in Kiruna. In addition, an extra nurse was appointed to the Kiruna child-welfare clinic. The local authorities and population of the two districts were prepared for the investigation through articles in the press and through personal contact.

#### 4. Antenatal Care

Attempts were made to come into contact with expectant mothers as early as possible during the pregnancy. They were interviewed at maternity-welfare clinics or by child-welfare staff. Each mother was then seen regularly at the maternity-welfare clinic or by her own doctor. At an early stage the child-welfare organization took a family history and dietary history in accordance with a special form.

As has already been mentioned, the dietary habits of the population of Norrbotten are on the whole satisfactory, and no true malnutrition, either quantitative

or qualitative, occurs in any section of the community. Nonetheless, the importance was stressed to all expectant mothers of fortifying the diet throughout the pregnancy with animal protein, iron, and vitamins. Iron preparations and multiple-vitamin preparations were provided free of cost from the time pregnancy was diagnosed until delivery.<sup>1</sup> Regular supervision of health as described above was aimed at, to provide as far as possible optimum conditions for the development of the fetus.

#### 5. General Health Supervision of the Children

The outline of the general health supervision, together with the times of the various immunizations, is shown in Fig. 4.

*Regular examinations.* Throughout the first year all children underwent regular, frequent examination. According to the original scheme, they were to be seen by

a doctor at about six-weekly intervals in addition to special examination in the event of serious disease. During the coldest winter periods, however, and during the spring, when roads often become impos-

<sup>1</sup> Vitamin capsules ('Multivimin') were generously provided by Astra, Ltd.

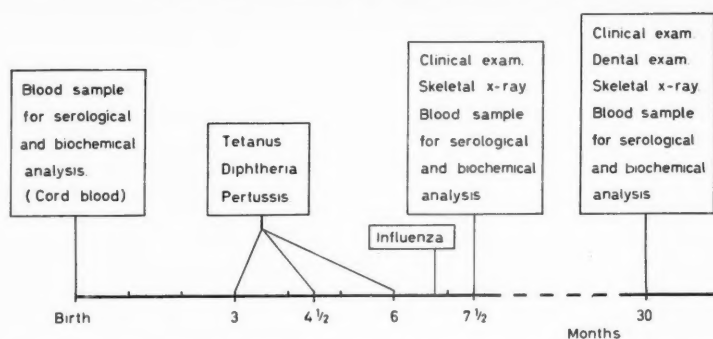


Fig. 4. Diagram to illustrate the time-table of the 'special examinations' and of the immunization procedures undertaken in the studies on antibody formation.

able for some weeks, this was not always possible. The nurses' visits to the children's homes were largely carried out according to plan, however, even under these difficult conditions, the children being seen at 2-weekly intervals. In Töre-Råneå the schedule was maintained throughout the first year of life; in Kiruna the interval between examinations after the special examination at '7½ months' became prolonged to 3-4 weeks. Careful notes on the child's health were made at every exami-

nation by doctor or nurse, on a special form. The mean number of examinations by physician or nurse is given in Table 7.

This intensified supervision was kept up throughout the first year of life. Subsequently the ordinary child-welfare-clinic pattern of supervision was followed, but with the same nurse as before. This was continued until the follow-up examination at '30 months' of age.

*Special examinations.* In addition to the regular examinations, special tests were

TABLE 7. Mean number of examinations by physician or nurse.

	Feeding groups			
	I	II	III	IV
<i>Kiruna</i>				
No. of examinations by physician	6.2	6.6	6.6	6.1
by nurse	8.3	8.6	8.7	8.6
Mean total number of medical examinations	14.5	15.2	15.3	14.7
<i>Töre-Råneå</i>				
No. of examinations by physician	4.1	3.9	4.1	4.2
by nurse	15.4	15.4	15.8	15.4
Mean total number of medical examinations	19.5	19.3	19.9	19.6

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

performed at certain times, in accordance with the following scheme.

*At birth.* Tests on umbilical-cord blood (chemical data, including sodium, potassium, calcium, phosphorus, alkaline phosphatase, and protein determinations, and electrophoresis; serological data, including antistreptolysin and antistaphylococcal titres and diphtheria and pertussis antibodies).

*'7½ months'* (90 % of the children were aged 6 to 9 months). Full clinical exami-

nation was carried out by a pediatrician, including X-ray examination of the bones (to exclude rickets and to determine the stage of development of ossification centres) and blood tests as above.

*'30 months'* (90 % aged 26 to 34 months). Full clinical examination, as at *'7½ months'*, was again carried out. Dental examination was made by a specialist. Further X-ray examination and blood tests, as above, were performed on part of the series.

## 6. Immunization Procedures

*BCG immunization* was carried out during the first week of life, and tuberculin testing at 3 months. Most of the children were again tuberculin tested at *'7½ months'* and at *'30 months'*.

*Combined immunization* (Pertussis, diphtheria, and tetanus) was carried out at about 3, 4½, and 6 months of age. The titres of pertussis and diphtheria antibodies were estimated at *'7½ months'* and, in part of the series, also at *'30 months'*.

An investigation so complicated as this can only be carried out with the full support of everyone concerned in it. This is, of course, particularly true of the families involved. The project was preceded by articles in the press and by personal interviews, and the staff of the child-welfare

*Vaccination against small-pox* was carried out as a rule in the second half year of life, at a mean age of 8½ months.

*Influenza immunization* with a single injection of influenza A and B vaccine was performed on a limited group of children, usually about 3 weeks before the *'7½-month'* special examination, when serum was collected for antibody titration.

clinics maintained constant contact with the participants. The spirit of co-operation was at all times good, and not least was the excellent collaboration between mothers and staff a source of great satisfaction.

## CHAPTER V

### MATERIAL

by GUNILLA CARLGREN, OLOF MELLANDER, TORE MELLBIN, and BO VAHLQUIST

#### 1. Selection of Participants

Registration of patients took place during the period June 1st, 1953, until December 31st, 1954. The investigation then proceeded until May, 1957. The number of children born in the two districts during the registration period was 660. Strenuous efforts were made to enrol as many of these children as possible. For various reasons, a considerable number of them remained outside the investigation, either because they were never enrolled in the

first place, or because they failed to keep up attendances. The commonest causes of non-participation are shown in Table 8.

The total number of children remaining in the investigation until its conclusion was only 402. Having regard to the main object of the work, namely, to compare the influence of different modes of rearing upon the health of the child, this can hardly have had any important effect upon the findings.

TABLE 8. *Number of participants in relation to live births.*

District	Total no. of children born alive June 1, 1953–Dec. 31, 1954	No. of participants	Non-participants					Lack of cooperation
			Died shortly after birth	Died during the study	Serious disease in child or mother	Moved from district	Other obvious reason <sup>a</sup>	
Kiruna	349	193	7	1	3	21	17	107
Töre-Råneå	311	209	4	1	6	21	13	57
Total	660	402	11	2	9	42	30	164

<sup>a</sup> Premature birth, language difficulties, etc.

#### 2. The Mode of Rearing

Preliminary reports from child-welfare clinics in the two districts had indicated that the distribution of cases among the various feeding groups (see below) was

likely to be satisfactory. The true incidence of the degree of breast feeding in the two districts is shown in Fig. 5.

In attempting to refine the main issue

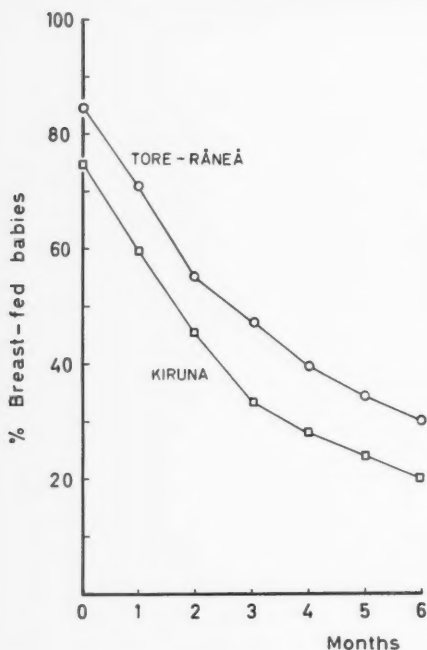


Fig. 5. The incidence of plain breast feeding during the first six months of life.

of the significance of the type of milk, it was necessary as far as possible to standardize the diet in other respects. The following scheme was adopted.

In cases where full breast feeding could not be achieved throughout the first 6 months, the feeds were supplemented with a 1:1 mixture of cow's milk and water with 5 % of sugar and 1 % of wheat flour. This mixture gives about 550 calories per litre, and contains about 1.7 % of protein, 1.5 % of fat, 8 % of carbohydrate, and 0.4 % of mineral substances. The maximum amount allowed per day was 600-700 g (21-25 oz) at one month, increasing to 800-1000 g (28-35 oz) at 3 months. Crushed rusks were added to one or two of the

feeds from 4 months. In one group the feed was prepared from fresh cow's milk, and in the other from a commercial dried-milk preparation giving a mixture of the same composition (cf. Gyllenswärd and Mellander, 1949).

The 'fresh milk' used in Kiruna was obtained from the shops, and had been pasteurized and the fat-content standardized to 3 % at the dairy. The interval between milking and consumption was as a rule about 2 days, but could in exceptional cases be as much as 3 days. Refrigeration was scrupulously maintained during transport and storage. In Töre-Râneă most families used fresh milk from local herds.

The diet was supplemented as follows. From one week 10 drops of a vitamin preparation were given daily, providing 2500 units of vitamin A and 1000 units of vitamin D. This dose was doubled from 3 months, from which time 25 mg of ascorbic acid was added per day, and also orange juice or infusion of the fruits of *Rosa canina*. Strained baby foods were introduced at 4 months.<sup>1</sup> After the special examination at '7½ months', the mothers were given a freer hand with the children's diets. During the period of the investigation no pressure was exerted on the mothers to breast feed their infants. The incidence of breast feeding was considerably higher in the rural district of Töre-Râneă than in the town of Kiruna, but any effect of this difference was eliminated by the statistical treatment (cf. Chapter VI).

Attempts were made to create, by the precautions just described and by the

<sup>1</sup> Strained baby foods were generously provided by Findus, Ltd.

prophylactic maternal welfare during pregnancy previously described, optimum conditions for the child's development, both in utero and during the first year of life. It is of course important to realize that conditions will in any event vary from case to case. The essential feature of the

investigation is that there were no systematic differences between the various groups, involving the mothers' health and diet or the family environment into which the children were to be born. Since this feature is of fundamental importance, it will be considered in some detail below.

### 3. The Four Feeding Groups

Different ways of arranging the series were first tried. The important point was to create two 'extreme' groups, one in

which the infants had been nursed exclusively at the breast for a very long time, and one in which they had been breast fed

TABLE 9. *Distribution into feeding groups.*

District	Feeding groups				I + II + III + IV
	I	II	III	IV	
Kiruna	79 41	52 27	32 17	30 15	193 100
Töre-Råneå	64 31	45 21	39 19	61 29	209 100
Total	143 35	97 24	71 18	91 23	402 100

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Figures in italics are percentages.

TABLE 10. *Duration of partial breast feeding.*

District	Duration, months	Feeding groups				I + II + III + IV
		I	II	III	IV	
Kiruna	Total	79 100	52 100	32 100	30 100	193 100
	0	16 20	3 5.5	2 6	1 3	22 11
	1	22 28	16 31	5 16	5 17	48 25
	2	21 27	14 27	6 19	6 20	47 24
	3-4	13 16	14 27	15 47	11 37	53 28
	5-6	4 5	3 5.5	3 9	7 23	17 9
	> 6	3 4	2 4	1 3	0 0	6 3
	M	1.9	2.4	2.9	3.1	2.4
Töre-Råneå	Total	64 100	45 100	39 100	61 100	209 100
	0	9 14	3 7	1 3	7 11	20 9.5
	1	13 20	12 27	11 28	23 38	59 28
	2	11 17	10 22	7 18	15 25	43 20.5
	3-4	22 34	11 24	15 38	12 20	60 29
	5-6	7 11	8 18	4 10	4 7	23 11
	> 6	2 3	1 2	1 3	0 0	4 2
	M	2.6	2.7	2.7	1.9	2.4

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Figures in italics are percentages.

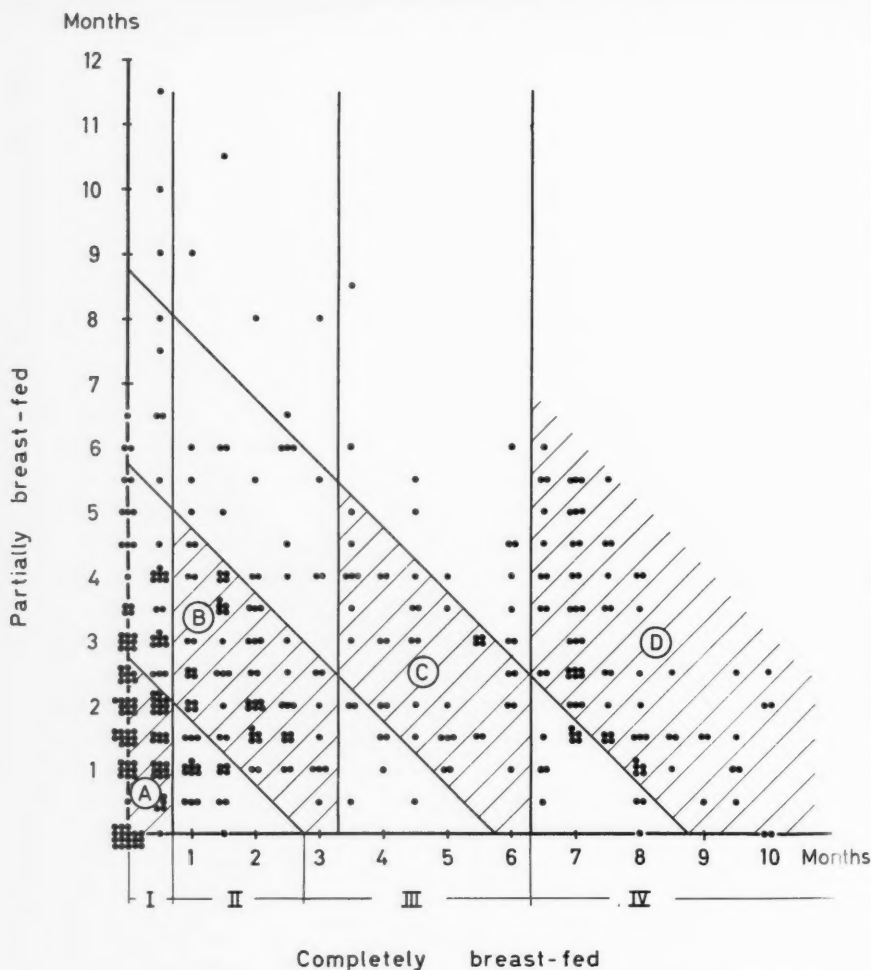


Fig. 6. Diagrammatic illustration of the two different modes of grouping the series.

I-IV. Standard grouping, with reference only to period of plain breast feeding.

A-D. Special grouping, with reference to both plain and partial breast feeding.

Each dot represents one infant, and the localization indicates the periods of plain and partial breast feeding.

for a very brief period. In addition, however, the many children who fell between these poles had to be placed satisfactorily. The simplest was to take account only of the period of plain breast feeding. This

method has usually been employed in earlier investigations, and the same principle has been adopted here. The series is divided into the following 4 groups.

- I Plain breast feeding 0-2 weeks  
 II " " " 1-2½ months  
 III " " " 3-6 months  
 IV " " " 6½ months or longer

The distribution of the individuals is shown in Table 9.

The duration of complementary feeding varied greatly between individuals. This is evident from Table 10 and Fig. 6.

It is clear that in arranging the series into 4 groups with different modes of rearing it would be desirable to take into account not only the duration of plain breast feeding but also the variable period of complementary feeding, and if possible also that quantity, small or large, of breast

milk consumed during the various stages. Obviously, a child fed solely at the breast for one month and to two-thirds during the next four months will receive more breast milk than an infant nursed at the breast alone for two months and to fifty per cent during only one month. A system of classification in which regard is paid to the duration both of plain breast feeding and of complementary feeding has been adopted in some preliminary reports (Vahlquist, Mellander, and Wicklund, 1957; Mellander and Vahlquist, 1957) and is also illustrated in Fig. 6. This is briefly discussed on page 87.

#### 4. The Families

It is clearly of decisive importance that the groups should not differ in any essential respect but the mode of rearing. Some of the data examined in this connexion are presented in the following. Details concerning the age of the mothers and the

housing of the families are given in Tables 11 and 12. Mean values only are given for certain other factors (Table 13). Concerning the dietary situation, the reader is referred to the next section.

As can be seen from the tables, the 4

TABLE 11. *Age of the mothers.*

District	Age	Feeding groups			
		I	II	III	IV
Kiruna	Total	79 100	52 100	32 100	30 100
	15-19	15	15	0	17
	20-29	60	70	59	47
	30-39	25	13	35	36
	≥ 40	0	2	6	0
	M, yrs	26.4	25.5	29.5	27.3
Töre-Råneå	Total	64 100	45 100	39 100	61 100
	15-19	3	9	5	3
	20-29	49	48	71	40
	30-39	41	35	24	44
	≥ 40	7	8	0	13
	M, yrs	30.1	29.2	27.0	31.4

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Figures in italics are percentages.

TABLE 12. *Persons per living-room (kitchen excluded).*

District	Persons per room	Feeding groups			
		I	II	III	IV
Kiruna	Total	79 100	52 100	32 100	30 100
	≤ 0.9	1	2	3	0
	1-1.9	36	20.5	28	17
	2-2.9	24	21.5	28	50
	3-3.9	25	39	19	23
	≥ 4	14	17	22	10
	<i>M</i>	2.7	3.0	2.8	2.8
Töre-Råneå	Total	64 100	45 100	39 100	61 100
	≤ 0.9	0	0	0	0
	1-1.9	23	16	36	29
	2-2.9	39	35	31	38
	3-3.9	22	22	15.5	15
	≥ 4	16	27	17.5	18
	<i>M</i>	2.8	3.1	2.6	2.7

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Figures in italics are percentages.

TABLE 13. *Some data concerning the families in both districts.*

All figures are mean values.

	I	II	Feeding groups		I + II + III + IV
			III	IV	
Age of mother, years	28.0	27.2	28.0	29.1	28.1
Weight of mother, kg	61.5	61.0	61.9	63.5	62.0
Mother's Hb < 70 %	15 %	18 %	14 %	12 %	14 %
No. of children during last 5 yrs.	1.5	1.5	1.4	1.4	1.43
No. of siblings of school age	0.35	0.32	0.44	0.80	0.44
Health of siblings good	88 %	92 %	77 %	85 %	86 %
Middle class and well-to-do	14 %	16 %	14 %	14 %	14 %
Annual income, Sw. crowns					
Kiruna	8300	9100	9550	7650	8700
Töre-Råneå	7200	7100	7300	6550	7000
No. of living-rooms	2.0	1.8	2.1	2.1	2.0
No. of persons per living-room	2.75	3.05	2.7	2.75	2.8

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

groups are in most respects well matched. There are a few differences that deserve comment. The number of siblings of school age is significantly higher in group IV

than in the other groups. This might be due to a covariation between milk production and fertility, and perhaps even to greater viability of the offspring. On the

other hand, it is striking that the number of children 'during the last 5 years' does not differ from group to group. From an environmental point of view, however, the immediate effect of the difference would have been anticipated to be that the individuals of group IV would be exposed to greater risks of infection than children in the other groups. As we shall see (Table 29), however, the incidence of certain types of acute infection actually observed was in fact lower in group IV than in groups I-III. Thus this incongruity has not created, but possibly reduced, the

true differences that do in fact exist between the groups.

The number of mothers in whom the haemoglobin was less than 70 % varies slightly from group to group, but taking into account errors of the method and scatter within the groups this has no statistical significance.

The declared, yearly incomes vary quite considerably between the groups (and are lowest in group IV). These figures cannot be taken as indicative of the actual standard of living, and too much significance should not be ascribed to the differences.

### 5. The Diet of the Expectant Mothers

At the first examination by the midwife, the mothers participating in the investigation were requested to be prepared to give information about the family's dietary habits, and were informed that a nurse would later call to ask for such information. In good time before this visit the women were given lists of the most im-

portant foodstuffs, to be filled in as consumed over a period of two weeks. Explanations were provided by the nurse. After they had been filled in, the lists were gone through point for point, and unclear or inadequate information completed. This work was found to be full of pitfalls, and depended in every case to some extent

TABLE 14. *Calculated amount of nutrient per 'standard subject' and day.*

Type of nutrient	Unit	Feeding groups			
		I	II	III	IV
Calories		3102	3214	3056	3053
Protein	g	69	74	71	70
Fat	g	165	169	155	152
Carbohydrate	g	297	310	306	312
Calcium	mg	1425	1622	1477	1474
Phosphorus	mg	1597	1722	1637	1642
Iron	mg	7.9	7.6	7.7	7.7
Vitamin A	I.U.	3043	3160	2876	2711
Thiamine	mg	1.13	1.16	1.14	1.16
Riboflavin	mg	2.1	2.2	2.1	2.2
Niacin	mg	6.8	6.9	6.8	6.7
Ascorbic acid	mg	32	33	32	32

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

TABLE 15. *Consumption of certain food constituents per 'standard subject' and day*

Type of food	Unit	Feeding groups			
		I	II	III	IV
Milk	litre	1.15	1.21	1.18	1.24
Cream	100 cc.	0.15	0.16	0.13	0.10
Cheese	kg	0.025	0.040	0.028	0.023
Butter	kg	0.054	0.050	0.045	0.050
Margarine	kg	0.049	0.050	0.050	0.040
Bread	kg	0.152	0.150	0.160	0.170
Sugar	kg	0.103	0.114	0.108	0.101
Potatoes	kg	0.300	0.300	0.300	0.300
Meat	kg	0.119	0.117	0.110	0.108
Fish	kg	0.070	0.060	0.076	0.060

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

upon the veracity and interest of the mothers. Naturally enough, many of them could not be got to persevere with the lists, and some gave answers that were so obviously untrue as to be worthless. Finally, 337 lists were acceptable.

The information has been analysed with respect to the size of the families and the age of the children, in conformity with the norms laid down by the Swedish Social

Welfare Bureau. The results are given in Tables 14 and 15.

Having regard to the great sources of error that characterize interview investigations of this nature, it may be stated that no essential differences exist between the 4 groups, and that no dietary factor was present in subnormal quantity. Possibly, the consumption of milk has been unusually high.

## 6. The Infants at Birth

Of the births 94 % took place in a special maternity clinic. In Kiruna, the only institution of this kind is at the hospital there. In Töre-Råneå there are 3 separate mater-

nity wards, all situated outside the actual district of the investigation. For practical reasons it was impossible to carry out any uniform or specialist examination of the

TABLE 16. *Distribution with respect to birth-place and sex.*

Sex	Kiruna				Töre-Råneå			
	I	II	III	IV	I	II	III	IV
♂	33 42	20 38	18 56	17 57	33 52	23 51	20 51	48
♀	46 58	32 62	14 44	13 43	31 48	22 49	19 49	52

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Figures in italics are percentages.

TABLE 17. *Birth weight.*

IV	Weight in kg	Feeding groups				I + II + III + IV
		I	II	III	IV	
	Total	143	97	71	91	402
0.24	≤ 2.4	2	3	7	1	11 3
0.10	2.5-2.9	20	17	7	13	60 15
0.023	3.0-3.4	40	30	32	38	143 36
0.050	3.5-3.9	25.5	37	41	34	134 33
0.040	4.0-4.4	11.5	12	10	13	48 12
0.170	≥ 4.5	1	1	3	1	6 1
0.101	M	3.38	3.46	3.50	3.49	3.45
0.300						
0.108						
0.060						

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Figures in italics are percentages.

newborn infants. In view of the object of the investigation, however, this was probably not a very serious disadvantage.

The sex-distribution of the children is shown in Table 16, the birth-weights in

TABLE 18. *Seasonal distribution of births.*

Season	Feeding groups			
	I	II	III	IV
Winter (Oct. 1 to Mar. 31)	68 48	33 34	32 45	46 48
Summer (Apr. 1 to Sept. 30)	75 52	64 66	39 55	45 52

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Figures in italics are percentages.

Table 17, and the time of year birth took place in Table 18. The few differences that do occur are in no case significant.

The series consists of 194 boys and 208 girls. Thus the girls show a slight numerical superiority, in contrast to the usual state of affairs. This difference is not statistically significant. Concerning the birth-weights, the infants of group I were slightly smaller than the others. The difference between groups I and IV (3.38 and 3.49 kg respectively) is not significant. With regard to the season of birth, rather more children were born during the period April 1st to September 30th than during the remainder of the year (223 and 179 respectively).

## CHAPTER VI

# STATISTICAL METHODS

by GUNNAR EKLUND

### 1. Significance Levels

In this monograph the term 'significant' has been used in accordance with the following convention.

If an observed difference between two percentages (or two means) is of a magnitude such that the probability  $P$  of obtaining a difference at least as great as the observed value is greater than 0.05 (where the null hypothesis is assumed to hold), then that observed difference is said to be *non-significant*.

If  $0.01 < P \leq 0.05$  the difference is said to be (*probably*) *significant* and is marked \*.

If  $0.001 < P \leq 0.01$  the difference is said to be *significant* and is marked \*\*.

If  $0.001 \geq P$  the difference is said to be (*highly*) *significant* and is marked \*\*\*.

Only two-tailed significance tests have been applied.

The significance test is performed either by using a normally distributed test variable or by applying a  $t$ -test or a binomial test.

### 2. Confidence Intervals

The confidence intervals reported in this monograph are symmetrical at the 95% level. The confidence limits are estimated by means of normal or binomial distributions.

The confidence intervals and the significant judgements which are found below the tables, refer either to the difference between a mean (or percentage) for group IV and a mean (or percentage) for group I, or to the difference between a mean for the groups IV and III taken together and the mean for group I. In the tables, mean values (percentages) are given for the infants belonging to these groups. In general the confidence intervals with the help of sample means supply information concerning the population means. The mean values given in the tables can be regarded as sample means. The assertion that the population difference between the means (percentages) for, say, groups IV and I lies *within the confidence interval* will be true, on the average, in 95% of instances, in which the assertion is made. It is here assumed that the samples are randomly drawn from the population.

Now we meet a complicated question. How is the *population* to be specified? It cannot be claimed that the children are drawn at random from a population consisting of all infants born in Norrbotten during 1953-1954. It is therefore preferred to specify some hypothetical population. It is then assumed that there is a very large population of children and from this the samples are considered to be drawn at random.

### 3. Incompleteness of Data

From Table 8 is seen that data are missing for a considerably large group (the uncooperative group). It must be admitted that this group can have a different composition from that of the remaining material.

In commenting on the tables it has been assumed that,

- (i) the values of the dependent variables (weight at 3 months, number of infective incidents etc.) have no effect on the frequency of cooperative mothers, e.g. the mothers of infected children cooperate with the same frequency as do mothers of uninfected children.
- (ii) there is no factor influencing both the dependent variable and the cooperation frequency. (Cf. Berkson, 1955).

These assumptions seem to be reasonable. In any case, it is difficult to give a concrete example of a biasing factor of (ii).

### 4. Intra-individual Comparisons

The majority of comparisons in this monograph are made between groups. Some comparisons, however, are intra-individual. The reason for this is illustrated by the following example.

We want to decide whether the caries activity is significantly greater or smaller in the teeth mineralized during the period of breast feeding than those mineralized during periods of artificial feeding. We can now compare the most extreme groups, IV and I, with respect to caries activity. This comparison is, however, inefficient since the dispersions are large: caries activity varies greatly. A considerably smaller dispersion is obtained if it

is based on intraindividual differences (see p. 69).

### 5. Notation and Comments on the Tables

Mean is symbolized  $M = \frac{\sum x_i}{n}$ , where  $x_i$  denotes the value (e.g. weight gain) for the individual ( $i$ ), and  $n$  the number of individuals.

Standard deviation (dispersion) is defined as

$$s = \sqrt{\frac{\sum (x_i - M)^2}{n}}$$

Mean error,  $s_M$ , is defined as  $s_M = \frac{s}{\sqrt{n-1}}$ .

The confidence interval for the difference between, say, groups IV and I is written (approximately) as

$$M_{IV} - M_I \pm 2 \sqrt{\frac{s_{IV}^2}{n_{IV}-1} + \frac{s_I^2}{n_I-1}}$$

Some of the means, standard deviations, and mean errors are *weighted*.

Thus for Tables 13, 17, 18, 19, 20, 21, 23, 24, 25, 29, 30, 36 and 37, the following *weighted* means are constructed.

$$M = \frac{M_{Kir} + M_{Töre-Råneå}}{2}, \text{ where } M_{Kir} \text{ and}$$

$M_{Töre-Råneå}$  are the simple means for the respective districts.

For these tables the standard deviation is calculated as

$$s = \sqrt{\frac{s_{Kir}^2 + s_{Töre-Råneå}^2}{2}}$$

The diagrams in Figures 10 and 11 are calculated in an analogous manner. The number of infants is not recorded for all tables. For such tables the distribution into feeding groups is the same as in Tables 9 and 19.

*Adjusted values.* The individual values for *weight*, *height*, and the number of *ossification centres* have been adjusted in order to approximate the weight etc. at '7½ m' and '30 m', since the measurements were not recorded at the same age for all infants. The adjustment norms

for weight and height are taken from Karlberg and Perman (1959), and for ossification centres from Elgenmark (1946). Say 13 ossification centres are recorded at 8½ months, then the adjusted number for '7½ m' will be 12.

## CHAPTER VII

### RESULTS

#### 1. Clinical Examination

by HERBERT ENELL, HELGE JOSEFSSON, OLLE LAGERSTAM, THOMAS LÄNNE, TORE MELLBIN, IVAN THORELL, BO VAHLQUIST, HARRIET WICKLUND, and PER ZETTERQVIST

The series comprises 402 infants. Some selection inevitably occurred, and the reasons for this are evident from Table 8, p. 43. Eleven infants with a birth weight of less than 2,500 g are included.

#### *Physical development. Weight, height, and ossification centres*

##### *Weight*

The children were weighed on standard infant scales. Different equipment was of course used at the different centres and the domiciliary visits, but the accuracy of the scales was checked at the beginning of the investigation. For practical reasons it was impossible to carry out weighing at the same time of day throughout.

*Results.* The figures given in Table 19 show the weight gain since birth after certain intervals up to '30 months'. Table 19 includes also the average birth weights for the various feeding groups. The initial loss in weight is not noted, but there was no significant difference between the groups. A diagram showing the weight gain from birth to '7½ months' is given in Fig. 7.

Comparison of the feeding groups re-

TABLE 19. *Weight gain since birth.*  
(Mean values and standard deviations in kilograms.)

Age	Feeding groups			
	I	II	III	IV
Mean				
Birth weight	3.38	3.46	3.50	3.49
Weight gain				
3 w.	0.24	0.29	0.38	0.41
1½ m.	1.00	0.99	1.12	1.26
3 m.	2.44	2.18	2.33	2.48
4½ m.	3.53	3.22	3.26	3.41
6 m.	4.40	4.19	4.06	4.16
'7½ m.'	5.52	5.25	4.97	4.90
'30 m.'	10.54	9.88	9.49	9.88
Standard deviation				
3 m.	0.60	0.55	0.56	0.52
'7½ m.'	0.98	1.07	0.84	0.89
'30 m.'	1.78	1.77	1.63	1.83
3 m. n	143	97	71	91
'7½ m.' n	143	97	71	91
'30 m.' n	128	79	61	85

*Feeding groups* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Confidence interval for the difference between the means for groups IV and III on the one hand and the mean for group I on the other: 3 months:  $-0.03 \pm 0.13$ .

Confidence intervals for the difference between the mean for group IV and the mean for group I: '7½ months':  $-0.62 \pm 0.25$  (significant\*\*\*)  
'30 months':  $-0.66 \pm 0.51$  (significant\*)

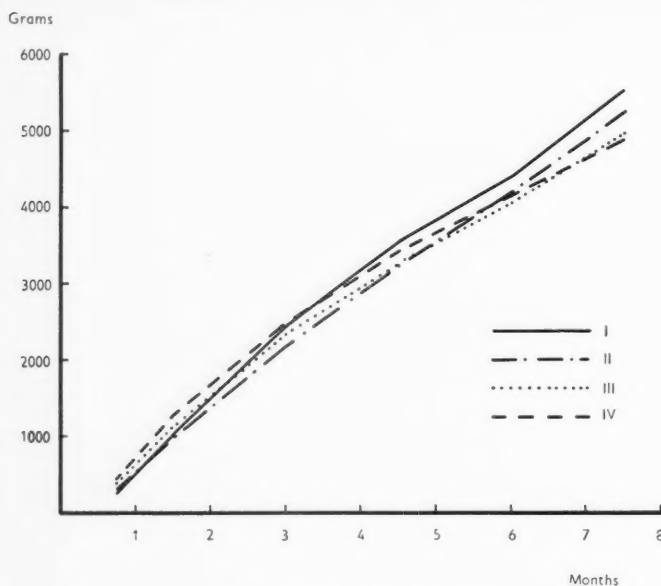


Fig. 7. Weight gain since birth.

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

veals that the average weight-gain of the infants in group I (weaned early from the breast) at '7½ months' is significantly (\*\*\*) higher than that of the infants in group IV.

*Discussion.* The results obtained indicate that temporary differences concerning weight gain may exist between breast-fed and bottle-fed infants during the first year of life, but that none are demonstrable at '30 months'. Earlier investigations have resulted in somewhat different findings (see Chapter I), but have on the whole suggested that the weight gain of bottle-fed infants is similar to or greater than that of breast-fed, at 6 months or over.

The difference with respect to weight gain may well be a result of differing daily

intake of calories. It is possible that this was on an average higher among the bottle-fed during the second trimester, when some of the breast-fed may have experienced some insufficiency but were nonetheless kept on the breast up to the 'ideal' time.

### Height

Infants aged less than one year were measured lying down on a measuring board with fixed foot block and movable head end, and older children standing up. Height was measured only at 'special' examinations at '7½' and '30 months'. For practical reasons it was impossible to perform the measurements uniformly at the same time of day.

*Results.* These are collected in Table 20. It can be seen that there is a small but

TABLE 20. *Height.*

	Feeding groups							
	I		II		III		IV	
	♂	♀	♂	♀	♂	♀	♂	♀
'7½ m.' mean	70.8	69.2	70.5	68.2	69.0	68.1	69.8	67.8
'30 m.' mean	90.4	89.0	91.1	88.4	89.4	89.3	90.3	88.1
'7½ m.' standard deviation	2.4	3.2	3.1	2.5	2.3	2.3	2.0	1.9
'30 m.' standard deviation	3.2	4.1	3.5	3.5	3.3	3.7	3.4	3.4
'7½ m.' <i>n</i>	66	77	43	54	38	33	46	45
'30 m.' <i>n</i>	60	68	34	45	32	29	41	44

Height figures in centimetres.

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Confidence intervals for the difference between the mean for group IV and the mean for group I:

'7½ months' ♂:  $-1.0 \pm 0.85$  (significant\*)

'30 months' ♂:  $-0.1 \pm 1.4$

'7½ months' ♀:  $-1.4 \pm 1.0$  (significant\*\*)

'30 months' ♀:  $-0.9 \pm 1.5$

significant difference (boys\*, girls\*\*) between groups I and IV at '7½ months', the bottle-fed infants being the taller. At '30 months' this difference is equated.

*Discussion.* As has been mentioned in Chapter I, the few earlier investigators who have measured the height of breast- and bottle-fed infants have found a similar difference at about 6 months of age, with slightly higher values for the latter, but no difference after the first year.

#### Ossification centres

The method originally described by Son-tag, Snell, and Anderson (1939) and later modified by Elgenmark (1946), involves counting all the ossification centres of the upper and lower limbs of one side. For technical reasons one centre (the coracoid) was not included in the present study. For the '7½ months' examination the infant was fixed to a special board, and at '30 months' the child was held still. The children from Töre Råneå were examined at the hospital at Boden (Dr. Ivan Thorell), and those from Kiruna at the hospital there (Consultant

Radiologist Dr. Einar Isberg). All films were read at the Department of Radiology, University Hospital, Uppsala (Professor Folke Knutsson), by Dr. Harriet Wicklund. Radiological examination of all infants was aimed at at '7½ months', but for practical reasons at '30 months' this was only possible in the case of the Kiruna children.

*Results.* These are collected in Table 21. It can be seen that among the girls there is a significant difference (\*\*) concerning development of ossification centres between groups I and IV at '7½ months', the bottle-fed (group I) showing the more rapid development. There is no significant difference among the boys. It is an interesting point that the well-known difference between boys and girls concerning the development of ossification centres appears at so early an age as '7½ months', with an average of 1.7 more centres among the girls.

*Discussion.* The means for the entire series are in accordance with Elgen-

TABLE 21. *Number of ossification centres.*

	Feeding groups							
	I		II		III		IV	
	♂	♀	♂	♀	♂	♀	♂	♀
'7½ m.' mean	12.3	15.0	12.5	14.3	12.6	13.3	12.0	13.6
'30 m.' mean	40.0	52.0	44.2	50.1	40.5	52.0	39.3	50.5
'7½ m.' standard deviation	2.1	3.7	1.9	3.8	1.9	2.4	1.5	2.0
'30 m.' standard deviation	6.5	4.5	3.5	4.0	6.6	4.9	4.7	6.9
'7½ m.' <i>n</i>	65	75	43	52	36	33	46	44
'30 m.' <i>n</i>	24	31	13	21	12	10	15	12

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Confidence intervals for the difference between the mean for group IV and the mean for group I,

'7½ months' ♂:  $-0.3 \pm 0.7$

'30 months' ♂:  $-0.7 \pm 3.7$

'7½ months' ♀:  $-1.4 \pm 1.1$  (significant\*\*)

'30 months' ♀:  $-1.5 \pm 4.5$

'7½ months' ♂ + ♀:  $-0.9 \pm 0.7$  (significant\*)

mark's (1946) findings, for both '7½' and '30 months'. Stewart and Westropp (1953) studied bone development by means of radiology of the hand, and noted no significant difference. As far as we know, no systematic investigation into the development of ossification centres in different feeding groups by the more exact method used in this study has previously been carried out. Striking and unexplained is the difference between the sexes.

It is noteworthy that the differences in nutrition that influence weight-gain only to a moderate extent, have shown an established effect upon skeletal development. The explanation of this may be that the artificial mode of rearing involves excessive administration of calcium, phosphorus, protein, and other substances (see Chapter II). Even if absorption of these substances may be *relatively* lower in the bottlefed infant than in the breast-fed, the *absolute* quantities taken up are often greater. The most obvious explanation of

the accelerated growth is probably that the skeleton is not entirely protected against the excess intake of protein and certain minerals that commonly occurs on feeding with cow's-milk mixtures, even when these are fairly dilute, as in the present investigation.

#### *Findings at routine examination*

A number of minor abnormalities were noted, concerning the skin, firmness of the flesh, superficial lymph nodes, stools, motor development, and general behaviour (crying, sleep, etc.). These were not regarded as sufficiently important or sufficiently suitable for objective assessment to motivate statistical analysis, however. Cases of infantile eczema were recorded, and the figures are shown in Table 22. There is no significant difference between the groups. This finding tallies with those of the better-documented earlier investigations (see Chapter I).

It is inherent in prophylactic medicine,

TABLE 22. *Incidence of infantile eczema.*

Degree of skin changes	Feeding groups			
	I n = 143	II n = 97	III n = 71	IV n = 91
Slight	25	25	11	24
Moderate	4	5	6	0
Pronounced	1	3	0	1

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

and especially in an investigation of this nature involving regular, frequent examination, that the likelihood of the appearance of serious deficiency states is very slight. In fact, we have on no occasion observed definite signs of qualitative or quantitative malnutrition that could reasonably be related to the diet. This is

exemplified by the following. Although about 3 % of the infants showed raised serum-alkaline-phosphatase values, in no case was there X-ray evidence of rickets, at either '7½' or '30' months.

#### *Haemoglobin*

The 'Sicca' technique was employed. This is based on colorimetric determination

TABLE 23. *Haemoglobin values.*

	Hb %	Feeding groups			
		I	II	III	IV
'3 months'	56-59	0	0	0	1
	60-69	8	11	4	3
	70-79	42	49	32	31
	80-89	35	31	52	49
	≥ 90	15	9	12	16
	<i>M</i>	81	79	83	83
'7½ months'	60-69	1	1	1	2
	70-79	32	33	34	29
	80-89	51	56	55	58
	≥ 90	16	10	11	11
	<i>M</i>	84	83	83	84
'30 months'	60-69	0	3	0	0
	70-79	18	22	10	18
	80-89	62	65	75	59
	≥ 90	20	10	15	23
	<i>M</i>	85	83	85	85

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Figures in italics are percentages.

Confidence interval for the difference between the means for groups IV and III on the one hand and the mean for group I on the other. '3 months':  $2.0 \pm 1.5$  (significant\*\*)

Confidence intervals for the difference between the mean for group IV and the mean for group I: '7½ months':  $0.0 \pm 1.6$

'30 months':  $0.0 \pm 1.9$

TABLE 24. *The incidence of infants with one or more pyrexial incidents ( $\geq 37.5^{\circ}\text{C}$  or  $100^{\circ}\text{F}$ .)*

	Feeding groups			
	I	II	III	IV
Infants with one or more pyrexial incidents	28 20	15 15	16 23	14 15
Infants with no pyrexial incidents	115 80	82 85	55 77	77 85

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Figures in italics are percentages.

Confidence interval for the difference between the percentage for group IV and the percentage for group I:  $-5 \pm 11$

of reduced haemoglobin. The instruments were calibrated before the start of the investigation, and once again during its course. The findings are given as a relative percentage, 100% corresponding to 14.7 g of haemoglobin per 100 ml of blood.

**Results.** These are given in Table 23. The haemoglobin was estimated at '3', '7½', and '30 months'.

It can be seen that the mean value for haemoglobin at '3 months' is significantly higher (\*\*) in groups IV and III than in group I. The difference is small, however. At both '7½' and '30 months' the haemoglobin levels are similar in all groups.

**Discussion.** In the past it was believed that bottle-fed infants had a greater propensity to develop anaemia than those reared at the breast. The reason for this assumption is difficult to see, since the prepared feed, whether deliberately enriched with iron or not, will as a rule contain considerably more of this mineral than the breast milk. The modern tendency to give solids from an ever earlier age results in an even greater intake of iron irrespective of the type of milk feed. It is hardly surprising that the haemoglobin values in

this investigation have been entirely similar in all four feeding groups.

### *Pyrexia*

The temperature was taken per rectum, as a rule each time the infant was seen by a nurse. In addition to this routine measure, the temperature was taken whenever the mother called in the nurse in the event of acute infection. The number of measurements of temperature therefore varied greatly from one child to another.

**Results.** At the start the following classification was employed. The number of pyrexial incidents (temperature  $\geq 37.5^{\circ}\text{C}$  or  $100^{\circ}\text{F}$ .) was placed in relation to the total number of occasions on which the temperature was recorded. This system was subsequently found to be impracticable for several reasons, and in the end the figures were divided into two groups, children that had on no occasion had pyrexia, and those that had shown one or more pyrexial incidents.

As can be seen from Table 24, no difference could be found between the various feeding groups, with regard to the number of infants with pyrexial incidents.

*Discussion,* see p. 65.

### Sedimentation rate

The erythrocyte sedimentation rate has for many years been used to complement the temperature, as an index of the effects of infection. Neither is specific, but among the factors that evoke an increase in temperature and sedimentation rate, the infections are predominant, and this is particularly true of the paediatric age group. In this investigation the sedimentation rate was estimated routinely at '3' and '7½ months'.

*Technique.* The sedimentation rate was determined by Ström's (1933) 'micro' method. Care was taken that the tubes were accurately calibrated, since even moderate differences in diameter may cause wide variations in the results.

The figures were read off after 1 hour. Ström's 'normal values' for women and children are given as 4-8 mm/hour, with 'limit values' for women at 9-12 mm/hr. As far as we know, no figures obtained by

this technique have been published for the ages concerned in this investigation. It is known that during the earliest period of life the sedimentation rate is very low. This is at least partly due to the physiologically high haematocrit readings. By 3 months of age, however, these have completely changed, and have reached the typical infantile level, which is lower than that in adults.

*Results.* These are given in Table 25, from which it can be seen that at '3 months' there are statistically significant differences between the feeding groups, groups IV and III showing significantly (\*\*\*) higher values than group I. At '7½ months' the differences are equated. The scatter is considerable in all groups (in calculating the confidence intervals, regard was not paid to the asymmetrical distribution about the mean occurring in biological series of this nature).

*Discussion.* The findings can hardly be explained by differences in the circumstances concerning infection. As will be clear from the following paragraph, the incidence of upper respiratory infection and acute diarrhoea showed only minor differences between the different feeding groups. It is also difficult to see why there is a difference at '3 months' but not at '7½'. Factors in the blood that influence the sedimentation rate include the haematocrit reading, which is not important in this connexion, and, more particularly, the plasma protein pattern. Under normal conditions this latter undergoes great changes during the earliest period of life, in connexion with the transition from intra-uterine to extra-uterine life. This well-known fact is also evident from data presented in a later section of this chapter (Table 36, p. 76), from which it is clear that different modes of rearing af-

TABLE 25. *Sedimentation rate, mm/hour.*

	Feeding groups			
	I	II	III	IV
<b>'3 months'</b>				
mean	10.1	8.6	8.0	6.0
n	137	92	65	86
standard deviation	7.3	6.6	6.2	3.4
<b>'7½ months'</b>				
mean	11.2	11.4	13.1	12.6
n	142	97	71	91
standard deviation	8.1	7.6	7.6	9.0

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Confidence interval for the difference between the means for groups IV and III on the one hand and the mean for group I on the other. '3 months':  $-3.2 \pm 1.5$  (significant\*\*\*)

Confidence interval for the difference between the mean for group IV and the mean for group I: '7½ months':  $1.4 \pm 2.3$

TABLE 26. *Antistreptolysin and antistaphylo-lysins titres at '7½ months'.*

		Feeding groups			
		I	II	III	IV
Antistreptolysin	<i>n</i>	24	21	14	18
	titres > 200,				
	%	7	20	19	12
Antistaphylo-lysins	<i>n</i>	98	68	45	71
	titres > 2.0,				
	%	8	4	0	6

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Figures in italics are percentages.

fect directly or indirectly the gamma-globulin fraction, which is significantly (\*\*\*) lower in group IV (late weaned) than in the others. These figures are too few to form the basis of a full discussion on the differences in sedimentation rate noted, however. Electrophoresis was not carried out at '3 months' and no figures are available for fibrinogen at any age. It is therefore fruitless to speculate on the causes. We can only publish the established facts.

#### *Antistreptolysin and antistaphylo-lysins titres*

*Technique.* The antistreptolysin titres were determined by Ipsen's method (1944), and the antistaphylo-lysins titres by Packalén and Bergqvist's (1947). The tests were performed at the time of the special examinations at '7½' and '30 months'. Owing to technical difficulties, much antistreptolysin material was lost. Some specimens were spoiled by the growth of Gram-negative bacilli. It is known that this may give falsely high titres (Packalén, 1948). Probably owing to the plastic material of which the storage vessels were made, troublesome turbidity developed in many other cases during the titration. This unfortunate 'selection' took place purely at random, and should not, therefore, influence the outcome of the comparison of the different feeding groups.

*Results.* These are collected in Table 26. There were no statistically significant differences between any of the feeding groups.

*Discussion,* see p. 65.

#### *Incidence of acute infections*

##### *Epidemic disease*

The cases of certain named infective disease recorded in connexion with the investigation are shown in Table 27.

*Results.* The number of cases is small. There is nothing to indicate a higher incidence in one or more of the feeding groups, with regard to infections either in particular or in general.

##### *Acute infections of the upper respiratory passages and digestive tract*

The commonest infections occurring during the first year of life are those that are localized to the upper respiratory passages. Formerly diarrhoea was also very common, but modern prophylactic measures have resulted in great improvement in the position.

TABLE 27. *Incidence of named infectious disease during the first 12 months of life.*

Diagnosis	Feeding groups			
	I n = 143	II n = 97	III n = 71	IV n = 91
Measles	6	11	4	2
Varicella	8	2	4	7
Rubella	0	0	1	1
Exanthema subitum (Roseola infantum)	8	7	2	3
Total	22	20	11	13

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

**Methods.** All infective incidents were recorded on the children's Health Cards. In most cases, symptoms were recorded at the routine examinations, which were carried out at frequent intervals (see Chapter IV). In a few cases the data were obtained from the history only. The types of illness considered statistically are rhinitis, cough, otitis media, upper respiratory infection with pyrexia, and acute diarrhoea. Clearly, with illnesses of this nature it is often difficult to distinguish between the diagnoses, and to know whether a particular child is suffering from a succession of different illnesses or from exacerbations of the same one. Cases showing symp-

toms of more than one of the types of illness named are included under more than one heading. No attempt has been made to assess the degree of severity and duration of the illness.

The observation period for this particular series extends from birth to 1 year. One of three theoretical relationships might be anticipated between the mode of rearing and incidence of infection: breast feeding might exert an influence while it was proceeding, or it might continue to act after its discontinuation, or it might have no influence. In order to take into account as many as possible of these considerations, and to

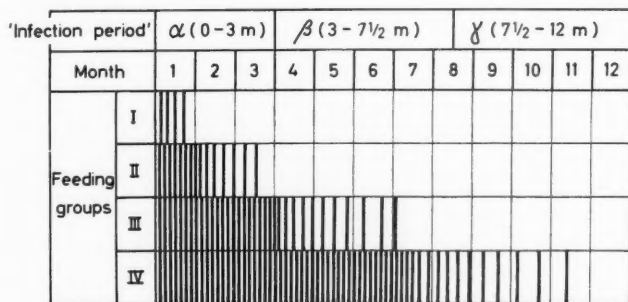


Fig. Diagram to indicate the successive decline in the number of completely breast-fed infants in the different feeding groups. The distribution into different 'infection periods' ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) is used in tables 28, 29 and 30 to compare the incidence of infective episodes.

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

TABLE 28. *Frequency of acute infections during the period 0-12 months. Mean value per infant.*

Diagnosis	Period	Töre-Råneå Feeding groups				Kiruna Feeding groups			
		I	II	III	IV	I	II	III	IV
Rhinitis	$\alpha$	0.69	0.59	0.62	0.41	0.27	0.35	0.27	0.27
	$\beta$	1.38	1.15	1.38	1.04	0.82	0.82	1.04	0.82
	$\gamma$	0.70	0.65	0.87	0.65	0.44	0.65	0.50	0.50
	$\alpha + \beta + \gamma$	2.77	2.39	2.87	2.10	1.53	1.82	1.81	1.70
Cough	$\alpha$	0.17	0.35	0.20	0.22	0.11	0.14	0.04	0.05
	$\beta$	1.03	0.87	0.78	0.69	0.71	0.53	0.85	0.35
	$\gamma$	0.42	0.30	0.42	0.30	0.41	0.41	0.27	0.22
	$\alpha + \beta + \gamma$	1.62	1.52	1.40	1.21	1.23	1.08	1.16	0.62
Otitis media	$\alpha$	0	0	0	0	0	0	0	0
	$\beta$	0.06	0.02	0.16	0.02	0.10	0.02	0.04	0
	$\gamma$	0.06	0.09	0.02	0.02	0.05	0.06	0.04	0
	$\alpha + \beta + \gamma$	0.12	0.11	0.18	0.04	0.15	0.08	0.08	0
Upper respiratory infections with pyrexia	$\alpha$	0.06	0.02	0.05	0.02	0.05	0.02	0	0.05
	$\beta$	0.45	0.35	0.47	0.39	0.23	0.18	0.23	0.11
	$\gamma$	0.38	0.20	0.22	0.39	0.20	0.14	0.19	0.14
	$\alpha + \beta + \gamma$	0.89	0.57	0.74	0.80	0.48	0.34	0.42	0.33
Acute diarrhoea	$\alpha$	0.06	0.02	0.11	0.07	0.03	0.06	0.04	0.05
	$\beta$	0.34	0.17	0.29	0.15	0.27	0.08	0.04	0.27
	$\gamma$	0.38	0.15	0.27	0.15	0.08	0.08	0.04	0.24
	$\alpha + \beta + \gamma$	0.78	0.34	0.67	0.37	0.38	0.22	0.12	0.56

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

$\alpha$ ,  $\beta$ ,  $\gamma$ : for definition see fig. 8.

utilize the material as fully as possible, the observation period was divided into three terms,  $\alpha$ ,  $\beta$ , and  $\gamma$ . As can be seen from Fig. 8,  $\alpha$  embraces the term 0-3 months,  $\beta$  3-7½ months, and  $\gamma$  7½-12 months. The average number of each type of infection per child over each term was calculated.

**Results.** The frequency of the 5 different acute infections during the 3 terms in the 2 districts is shown in Table 28. It is immediately obvious that the incidence of infection is higher in Töre-Råneå than in Kiruna, the only exception being the

middle-ear infections, which were relatively infrequent. What is the reason for the higher incidence of infection in Töre-Råneå? To some extent it may be due to the mode of presentation, and therefore be only ostensible. As has already been shown (Table 7, p. 41), the total number of medical examinations was higher per child in Töre-Råneå. Even though a history of infection was accepted, it is probable that this difference in frequency of examination may have been of some significance; apart from this the

TABLE 29. *Confidence intervals for differences between feeding groups.*

(cf. Table 28 and Fig. 8)

Pe- riod	Differences between the following 2 sets are calculated			Rhinitis	Cough	Otitis media	Upper respi- ratory infec- tion with pyrexia	Acute diarrhoea
	1st set	2nd set						
Töre-Råneå								
$\alpha$	III + IV	I		$-0.18 \pm 0.25$	$0.04 \pm 0.13$	—	$-0.03 \pm 0.07$	$0.03 \pm 0.09$
$\beta$	IV	I + II		$-0.24 \pm 0.35$	$-0.27 \pm 0.36$	$-0.03 \pm 0.06$	$-0.02 \pm 0.21$	$-0.12 \pm 0.15$
$\gamma$	IV	I + II + III		$-0.09 \pm 0.26$	$-0.09 \pm 0.22$	$-0.04 \pm 0.05$	$0.12 \pm 0.20$	$-0.13 \pm 0.14$
Kiruna								
$\alpha$	III + IV	I		$0.00 \pm 0.17$	$-0.06 \pm 0.10$	—	$-0.02 \pm 0.07$	$0.02 \pm 0.08$
$\beta$	IV	I + II		$0.02 \pm 0.28$	$-0.29 \pm 0.26^*$	$-0.07 \pm 0.06^*$	$-0.07 \pm 0.16$	$0.08 \pm 0.18$
$\gamma$	IV	I + II + III		$0.07 \pm 0.31$	$-0.16 \pm 0.20$	$-0.05 \pm 0.04^*$	$-0.04 \pm 0.15$	$0.17 \pm 0.17^*$
$(\text{Kiruna} + \text{Töre-Råneå}) \cdot \frac{1}{2}$								
$\alpha$	III + IV	I		$-0.09 \pm 0.15$	$-0.01 \pm 0.08$	—	$-0.02 \pm 0.05$	$0.02 \pm 0.06$
$\beta$	IV	I + II		$-0.11 \pm 0.22$	$-0.28 \pm 0.22^*$	$-0.05 \pm 0.04^*$	$-0.04 \pm 0.13$	$-0.02 \pm 0.11$
$\gamma$	IV	I + II + III		$-0.01 \pm 0.20$	$-0.13 \pm 0.15$	$-0.04 \pm 0.03^*$	$0.04 \pm 0.12$	$0.02 \pm 0.11$

differences must be true ones. It may be mentioned that housing conditions are altogether much poorer in Töre-Råneå, which is a country district.

The different incidence of infection in the two districts does not affect the main point of the investigation, the comparison of the different feeding groups, but it was thought best to describe separately the findings from the two districts.

A survey of the differences in frequency of infection between different feeding groups is given in Table 29. The grouping in this table is slightly different from that used in the rest of the investigation, in order to bring out as clearly as possible the differences between breast- and bottle-fed infants during different terms (cf. Fig. 8).

It is evident from Table 29 that during term:  $\beta$  (3–7½ months) and  $\gamma$  (7½–12

months) there exist significant (\*) differences, the values throughout being higher for bottle-fed infants, but these are often small, and never exceed the 5 % level.

The question of susceptibility to infection is approached from another angle in Table 30. Here the incidence of different types of infection during the various 'infection terms' ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) is calculated. The figures given in table 30 therefore take no account of the fact that the individual infant may have suffered from repeated attacks of the same type of infection during the period in question. The results reveal that for the combined periods  $\alpha + \beta + \gamma$  (0–12 months) the infants of group I (weaned early from the breast) showed significantly (\*\*) higher values than those of group IV (late weaned).

*Discussion.* Up to the 1930's most investigations showed a considerably higher

TABLE 30. Incidence of different types of infection during the different 'infection periods' ( $\alpha$ ,  $\beta$ , and  $\gamma$ ).

Feeding groups					Feedings group				
I	II	III	IV		I	II	III	V	
Number of children					Number of children				
143 100	97 100	71 100	91 100		143 100	97 100	71 100	91 100	
$\alpha$ -period					$\gamma$ -period				
0	84 59	60 62	44 62	59 65	0	50 35	42 43	27 38	31 31
1	38 27	24 25	14 20	16 18	1	37 26	20 21	19 27	32 35
2	18 13	12 12	12 17	15 16	2	36 25	23 24	18 25	22 24
3	3 2	1 1	1 1	0 0	3	18 13	9 9	7 10	6 7
4	0 0	0 0	0 0	1 1	4	2 1	3 3	0 0	0 0
5	0 0	0 0	0 0	0 0	5	0 0	0 0	0 0	0 0
Mean	0.58	0.53	0.58	0.55	Mean	1.20	1.08	1.07	1.03
$\beta$ -period					$\alpha+\beta+\gamma$				
0	27 19	21 22	13 18	21 23	0	12 8	10 10	4 6	10 11
1	38 27	26 27	19 27	21 23	1	17 12	14 14	12 17	18 20
2	47 33	37 38	23 32	33 36	2	39 27	37 38	22 31	31 34
3	23 16	12 12	8 11	16 18	3	54 38	28 29	21 30	23 25
4	6 4	1 1	8 11	0 0	4	17 12	7 7	11 15	7 8
5	2 1	0 0	0 0	0 0	5	4 3	1 1	1 1	2 2
Mean	1.64	1.44	1.70	1.48	Mean	2.41	2.11	2.37	2.05

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

$\alpha$ ,  $\beta$ ,  $\gamma$ : for definition see fig. 8.

Figures in italics are percentages.

Confidence intervals for differences between the means for the following groups.

Period  $\alpha$ : Groups III and IV on the one hand and group I on the other:  $-0.02 \pm 0.13$ .

Period  $\beta$ : Group IV on the one hand and groups I and II on the other:  $-0.08 \pm 0.16$ .

Period  $\gamma$ : Group IV on the one hand and groups I, II and III on the other:  $-0.10 \pm 0.14$ .

Periods  $\alpha + \beta + \gamma$ : Group IV on the one hand and group I on the other:  $-0.36 \pm 0.22$  (significant \*\*).

incidence of infection in infants reared by the bottle. Critical analysis of these studies, however, clearly reveals that poor standards of hygiene and concomitant increased risks of infection were of great significance in the case of these infants, and it is therefore difficult to determine what effect the differences in composition of the artificial milk mixtures and breast milk may themselves have had. Recent investigations, in which more attention has been paid to these sources of error, have still in most cases shown a preponderance of acute infections in bottle-fed infants, es-

pecially with regard to upper respiratory infection and acute diarrhoea, but the differences have often been only moderate (see Chapter I).

In the investigation now presented in which very great care was taken to ensure that the different feeding groups were closely similar with regard to factors other than the nature of the milk, the differences obtained never exceeded the 5% level. The general tendency, however, has been that early-weaned infants (group I) have shown a higher average incidence of infection than the late-weaned (group V).

By means of a special classification (Table 30), in which the incidence of different types of infection involving the upper respiratory passages and the gut were compared, a significant (\*\*) difference was obtained.

It should be mentioned that the risk of contracting infection may have been somewhat higher for the infants in group IV, since the number of siblings of school age in this particular group is higher than in the other groups (see Table 13). This fact further emphasizes the differences.

Concerning named infective disease, the total number of cases was too few to be of any use in a statistical analysis, but there is no evidence of any difference between the feeding groups.

In this connexion it is worthy of note

that although the figures for pyrexia, sedimentation rate, and antistreptolysin and antistaphylolysin titres showed no significant differences between the feeding groups there is in fact a difference concerning the gamma globulin, the values for groups I, II, and III being significantly (\*\*\*) higher than that for group IV (see Table 36). This observation may perhaps be interpreted as indirect evidence that differences do indeed exist between the feeding groups concerning infection.

Statistical treatment of the results of the present investigation has been undertaken with regard only to the incidence of acute infections. Assessment of the severity and duration of the illnesses is impossible with the data available.

## 2. Dental Examination

by JOHN HEDLIN and CURT SJÖBERG

### *Deciduous teeth. Time of eruption, and incidence of caries*

#### *Time of eruption*

A tooth was regarded as having erupted as soon as any part of it was visible. Up to one year, the number of erupted teeth was recorded at short intervals in all infants at the regular examinations by doctor or nurse. After one year these records were maintained with the same exactitude in only a section of the series, mostly enrolled during the latter part of the investigation.

*Results.* These are collected in Table 31. Since there was no great difference between the two districts, the findings for the entire series are given together. The

figures for the second premolars are not given, because these teeth had not always erupted at the time of the last examination.

The teeth erupted in the usual order, viz., -01-, +01+, +02+, -02-, +04±, +03±, and +05±.

Comparison of the various feeding groups reveals only small numerical differences, none of which is statistically significant.

*Discussion.* Few investigations have been devoted to the relationship between the mode of rearing and the time of eruption of the teeth. Certain recent studies (see Chapter I) have led to the same con-

TABLE 31. *Eruption of deciduous teeth, average age in months.*

Feeding groups								
I			II		III		IV	
43			22		22		29	
No. of children examined								
Tooth-pair	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
+ 04 +	15.5	2.5	16.0	3.9	15.3	1.7	16.5	3.7
+ 03 +	18.9	3.5	18.9	3.0	17.7	2.9	18.6	3.1
+ 02 +	9.9	3.3	10.6	2.6	10.4	2.2	10.9	2.2
+ 01 +	9.1	2.6	9.8	2.5	9.5	1.7	9.5	2.0
− 04 −	15.4	2.5	15.9	2.8	15.7	2.4	16.7	3.5
− 03 −	19.2	3.8	19.1	2.7	18.8	2.4	19.1	4.1
− 02 −	11.9	4.3	12.8	4.0	12.5	3.2	12.3	3.1
− 01 −	6.9	3.2	8.5	3.4	7.7	1.5	7.8	1.9

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

clusion as we have drawn, namely, that the mode of rearing makes no important difference.

#### *Incidence of caries*

*Material.* The incidence of caries was recorded in great detail at the special examination at '30 months'. Some wastage of the original 402 cases resulted from removal from the district, and some of the parents refused to co-operate. Serviceable data were obtained from 144 children in Kiruna and 169 in Töre-Râneå, making a total of 313 cases.

*Methods.* Each child was examined by two different dental surgeons (J. H. and C. S.), both of whom have had many years experience of both adult and child patients. The examinations were carried out in Kiruna and at 4 different places in the Töre-Râneå district. The mirrors used were of type K.K.4, and the probes S.S.W. no. 5, in a few cases complemented by other probes (D.A.B. no. 2). A Castle 'Panovision' lamp was used.

The teeth were dried by means of compressed air, and cleansing was done where necessary with cotton wool and cellulose wadding. X-ray examination was not carried out.

The extent of the caries was registered in accordance with the Sundvall-Hagland system (1955), thus: Class A, commencing enamel caries, with decalcification but no actual defect; Class B, enamel caries with defect; Class C, enamel and dentine caries. In the final analysis of the material, regard is paid only to classes B and C, over which the findings of the two dental surgeons were in close agreement.

The flourine content of the drinking water is about 0.1 mg per litre in both districts.

*Results.* Two groups of tooth-surfaces were compared with regard to the total incidence of caries (Table 32). They represent regions particularly disposed to develop caries, and the mineralization of which takes place during fairly well de-

TABLE 32. *Time of mineralization of the enamel in the two groups of tooth surfaces selected in the study of caries frequency.*

Group A (20 surfaces)	
01 mesial surface	0-1.75 mths.
01 distal surface	0-1.75 "
02 mesial surface	0-2.0 "
02 distal surface	0-2.0 "
05 occlusal surface	0-2.5 "
Group B (16 surfaces)	
04 mesial surface	1.5 -4.0 mths.
04 distal surface	1.5 -4.0 "
02 buccal surface	2.0 -3.0 "
01 buccal surface	1.75-2.5 "

finished, different periods during the first six months of life.

There were certain differences between the two districts of the investigation. The Kiruna children of feeding groups I, II, and III showed a lower incidence of caries than the corresponding Töre-Råneå children. Since the main question referred to

the relationship between the different feeding groups, and because this was identical in the two districts, the figures for the combined series only are shown in Table 33.

Since the state of the teeth is markedly influenced by 'external' factors (caries-provoking diet etc.), even during the period 6 months-2½ years, the scatter in the series is very great. The differences found between the different feeding groups with regard to each of the groups of tooth-surfaces (group A and group B) are non-significant.

More reliable information about possible 'internal' effects upon mineralization due to different modes of rearing during the first period of life is obtained if instead the intra-individual differences between A and B are calculated. The feeding groups that should be chosen for comparison are II and IV. The infants of group II (plain

TABLE 33. *Incidence of caries. Comparison at '30 months' of two groups of tooth surfaces with different mineralization times.*

		Feeding groups			
		I	II	III	IV
		n = 112	n = 70	n = 52	n = 79
Group A	Mean	5.92	5.24	5.59	6.70
	Standard deviation	5.1	4.7	4.8	5.7
Group B	Mean	2.36	2.53	2.40	2.69
	Standard deviation	3.3	3.7	2.9	3.4
Intra-individual differences between Group A and B	Mean	3.56	2.72	3.18	4.01
	Standard deviation	3.7	3.3	3.4	3.9

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

Group A and Group B, see table 32.

Confidence intervals for the difference between the mean for group IV and the mean for group I.

A 0.78 ± 1.60

B 0.33 ± 0.98

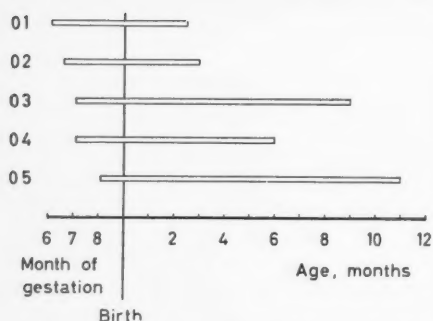


Fig. 9. Period during which the enamel structure of the deciduous teeth undergoes mineralization.

breast-feeding 1-2½ months) were largely fed solely at the breast during the period that the group-A surfaces were undergoing mineralization, but largely by the bottle when the group-B surfaces were being mineralized. The infants of group IV were essentially fed at the breast throughout the period of mineralization of both groups A and B. The difference between groups II and IV thus calculated is  $2.72-4.01 = -1.29^*$ .

*Discussion.* An important part of the mineralization of the teeth takes place during the first six months of life, that is, the nursing period. This is illustrated diagrammatically in Fig. 9, which is based on data published by different investigators (Bustin, Leist, and Priesel,

1929; Hess, Lewis, and Roman, 192; Logan and Kronfeld 1933; and Scheur and Massler, 1940). The consumption of minerals and protein is as a rule much higher on artificial feeding than with breast feeding, and this was true in the present investigation (cf. Table 6, p. 15). Probably a consequence of differences concerning mineral and protein intake, it has been demonstrated above that certain differences in skeletal development (height, development of ossification centres) exist between infants weaned early and late from the breast. It would seem reasonable to assume that the development of the milk teeth should also be affected by the composition of the diet during the first six months of life.

Several investigations have been devoted to the possible relationship between the incidence of breast feeding and the incidence of caries. A brief review of the literature has been published by Finn (1952). It is clear from this that opinions are conflicting. The results now presented suggest that the 'internal' effect of the type of milk upon mineralization tends to accord the bottle-fed infants a propensity. The difference does not exceed the 5% level, however. No attempt was made separately to assess the degree of severity of the caries.

### 3. The Antibody Response to Immunization

by LEO HELLER, GUNNAR LAURELL, TORE MELLBIN, BO VAHLQUIST, and IER ZETTERQVIST

*1. Collection of samples.* Blood samples for serological and chemical investigation were taken from mother and infant at delivery (from the cubital and umbilical veins,

respectively), and from the infant again at '7½' and '30 months' (from the femoral vein and the warmed finger tip, respectively). The blood was collected in specially cleaned

centrifuge tubes, which were filled to about  $\frac{3}{4}$ , stoppered, and allowed to stand at room-temperature for about 4 hours to coagulate. The tubes were then centrifuged for 10 minutes, and the serum removed with a separate pipette for each sample. Attempts were made to obtain 4-5 ml of serum, but this was often impossible. The serum was transferred to a 20-ml vessel of plastic material engraved with an identity number and equipped with a stopper coloured according to the group. (N.B. in a subsequent part of the investigation the serum was pipetted direct into two different small tubes.) The specimens were frozen immediately to  $-20^{\circ}\text{C}$ , and transported to the laboratory in Uppsala and subsequently to Gothenburg in a special container packed with carbon-dioxide snow. The serum was pipetted off in Uppsala for serological investigation, which was carried out at the University Institute of Hygiene and Bacteriology under the direction of Dr. Gunnar Laurell. The only serological procedure performed elsewhere was titration for influenza antibodies, which was done at the National Bacteriological Laboratory, Stockholm, by Dr. Leo Heller. The interval between sampling and analysis varied, but seldom exceeded 6 months. In the deep-frozen state the serum levels of antibodies and electrolytes remain unchanged for very long periods.

For obvious reasons, the volume of the serum samples varied greatly, and it was therefore impossible in many cases to carry out all the desired tests. Preference was given to the serological investigations, and these are consequently more fully represented than the chemical determinations.

2. *Combined immunization against pertussis, diphtheria, and tetanus* was carried out with vaccine from the National Bacteriological Laboratory, Stockholm. Each ml of this preparation contains 20-25,000 million B. pertussis killed by merthiolate, 12 units of diphtheria toxoid, and 7.5 units of tetanus toxoid, adsorbed to alu-

minium phosphate. The vaccine also contains an antiseptic and a quick-acting local anaesthetic (lignocaine).

Immunization was carried out in the manner usual in Sweden, 3 injections of 1 ml being given at about 3, 4 $\frac{1}{2}$ , and 6 months of age. The injections were given subcutaneously over the trapezius muscle on alternate sides. Blood samples were taken at two special examinations, at 3-6 weeks and at about 1 $\frac{3}{4}$ -2 $\frac{1}{4}$  years after the completion of immunization. During the period of the investigation two different preparations of vaccine were used (nos. 5 and 8). The antibody response to pertussis and diphtheria was studied. The effects of the two vaccines were slightly different, but both were fully satisfactory (cf. Laurell, Mellbin, Rabo, Vahlquist, and Zetterqvist, 1957). The results of immunization with vaccine no. 5 only are given below.

### *Diphtheria*

The titrations were carried out by the neutralization test in rabbits, as described by Jensen (1933). The individual values are the means of the two extremes at which the reaction changed from negative to positive.

*Results.* The antibody responses to vaccine no. 5 recorded in the various feeding groups at '7 $\frac{1}{2}$ ' and '30 months' are given in the form of cumulative frequency diagrams in Fig. 10. It can be seen that the findings for the various groups are very similar. No significant difference could be demonstrated between the groups in any case, either with the no. 5-vaccine alone or for the combined series with both vaccines.

The titres recorded after 3 injections must be regarded as fairly satisfactory.

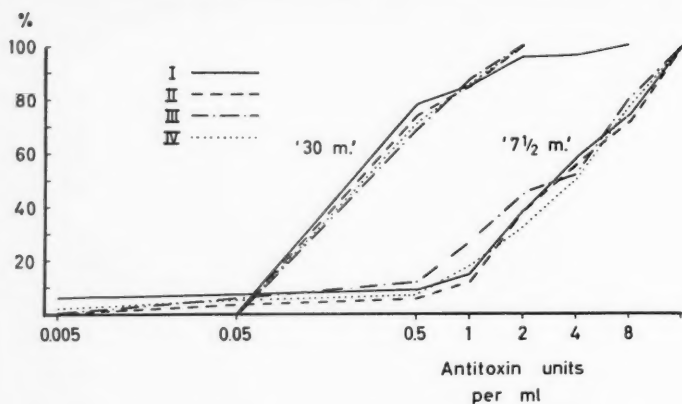


Fig. 10. Diphtheria immunization. Antitoxin titres shown in cumulative frequency curves.

	No. of children examined			
	Feeding groups			
	I	II	III	IV
'7 1/2 months'	69	50	40	60
'30 months'	27	27	16	35

Feeding groups. I birth-2 weeks, II 1-2 1/2 months, III 3-6 months, IV 6 1/2 months or more of plain breast feeding.

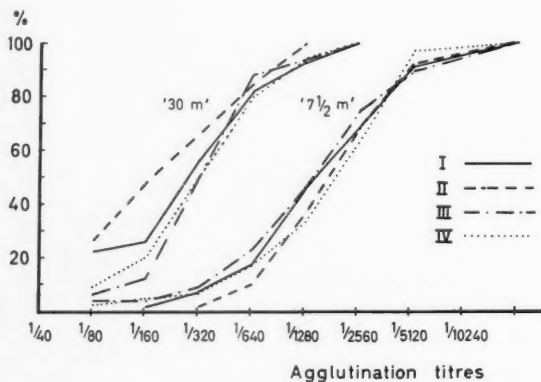


Fig. 11. Pertussis immunization. Agglutination titres shown in cumulative frequency curves.

	No. of children examined			
	Feeding groups			
	I	II	III	IV
'7 1/2 months'	69	50	40	60
'30 months'	27	27	16	35

Feeding groups. I birth-2 weeks, II 1-2 1/2 months, III 3-6 months, IV 6 1/2 months or more of plain breast feeding.

TABLE 34. *Immunization with Influenza B virus. Antibody titres (haemagglutination-inhibition test) following a single injection of the vaccine.*

Class	Titre level	Feeding groups			
		I	II	III	IV
0	1	4	3	2	5
1	1/64	2	—	—	—
2	1/91	3	2	2	1
3	1/128	3	2	—	2
4	1/181	2	3	—	2
5	1/256	—	2	—	2
6	1/362	—	—	1	—
7	1/512	2	—	3	2
8	1/724	—	—	—	—
9	1/1024	—	—	1	1
10	1/1448	—	—	—	1
11	1/2048	—	1	—	—
Mean titre class		2.44	3.31	4.44	3.69

*Feeding groups.* I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

The mean level for the entire series was at '7½ months' 3.0 units/ml, and at '30 months' 0.25 units/ml.

### *Pertussis*

*Methods.* The agglutination test was used for antibody titration. The tubes were incubated at +37°C for 1 hour, and read after being stored for a further 18 hours at +4°C.

*Results.* The findings are again given in the form of cumulative frequency diagrams (Fig. 11), in which account is taken only of the results obtained with vaccine no. 5. At '7½ months' there is no difference between the feeding groups; at '30 months' the figures for group I (early weaned) are slightly lower than those for group IV (late weaned), but the difference is insignificant. This is also true of the entire series, in which both vaccines nos. 5 and 3 were used.

The pertussis titres resulting from 3 inoculations of combined vaccine are highly satisfactory, with a median of between

1/1280 and 1/2560 at '7½ months' and at '30 months' 1/160 and 1/320.

3. *Immunization against influenza.* The vaccine was prepared at the National Bacteriological Laboratory, and contained influenza A (A-prim, A-SWE-3/50) and B (Lee). It was administered intracutaneously in a dose of 0.1 ml. For certain reasons it was only possible to immunize a small section of the series. The number of serviceable samples amounted to 54.

The titrations were performed by the technique described in the Bulletin of the World Health Organization (1953: 8: 5-6). The sera were pretreated with cholera enzyme. The titres indicate the dilutions of the serum in the last tube showing inhibition.

The results are shown in Table 34. Titrations were made against both A and B, but serological response was only obtained to B. There is a numerical difference between the different feeding groups but the series is too small to permit statistical assessment.

*Discussion.* Malnutrition may weaken the antibody response to immunization. This has been seen both in animal experiments (Cannon, 1949) and in clinical studies (Gell, 1951). The most obvious explanation would be the protein deficiency, but vitamin deficiency (Axelrod, 1958) and possibly other dietary factors may influence the response. It should be noted that impaired antibody formation is not a constant phenomenon in malnutrition (Balch, 1950; Gell, 1951).

In investigations in which impaired antibody response has been observed in states of protein deficiency, there has usually been severe shortage of animal protein in the diet. Moderate quantitative variations do not seem to have an established effect on antibody formation (Dancis, Osborn, and Julia, 1953).

A matter of considerable importance in young individuals is the question of whether the nature of the protein, species-specific or species-non-specific, is relevant. During this early period of life, the species-specific milk is the natural food, but human infants are often reared on the milk of some other animal species. Öberg and Mellander (1955) have shown in a series of animal experiments that the antibody response was better in goats fed on goat's

milk than in siblings fed on milk from another species. The difference was clearly apparent after immunization with influenza-A vaccine, but was not established after diphtheria immunization.

The results described in this chapter show no difference in antibody response to diphtheria and pertussis in the 4 feeding groups. With respect to influenza, the series does not permit statistical assessment.

It is possible that minor differences between the different feeding groups are suppressed under the influence of gross antigenic effect leading in all groups to a maximum or near-maximum antibody response. If this had been the case at '7½ months' a difference ought to have been present at '30 months', when the antibody titres had fallen markedly. This was not found, however. We can therefore only state that in our series there was nothing to indicate that the mode of rearing had influenced the antibody response.

In addition to the immunizations above named, BCG and smallpox vaccinations were performed routinely in most of the infants. Since several different doctors and nurses had been responsible for carrying out these procedures and checking the reactions, the material is not suitable for statistical analysis.

#### 4. Biochemical Analysis of the Blood

by OLOF MELLANDER, RAGNAR ÖSTERBERG, and LARS SVENNERHOLM

The samples of serum, taken as described in the preceding section (p. 70), were frozen and sent to Uppsala. The portion required for serological investigation

was pipetted off, and the remainder refrozen and sent to Gothenburg for chemical analysis. In the case of the '30 months' samples, thawing and refreezing were un-

TABLE 35. *Number of biochemical tests performed.*

	Maternal blood				Umbilical-vein blood				'7½ m' blood				'30 m' blood			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Total number of children in the study	143	97	71	91	143	97	71	91	143	97	71	91	56	45	30	38
No serum available for any biochemical test	48	39	22	24	54	46	27	29	30	19	16	14	0	0	0	0
Excluded owing to unreasonably high or low figures for sodium or calcium	14	11	9	15	10	3	3	7	7	1	1	4	0	0	0	0
Tests																
Total protein	74	42	38	46	63	42	39	52	105	76	54	73	56	45	30	38
Serum electrophoresis <sup>1</sup>	95	58	49	67	89	51	44	62	113	78	55	76	56	45	30	38
Phosphorus and alkaline phosphatase	73	40	37	41	60	37	36	47	99	71	54	69	37	32	18	31
Calcium	71	37	36	36	50	26	27	39	85	59	38	54				
Sodium and potassium	61	29	32	33	36	20	19	35	63	46	25	40				

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

<sup>1</sup> The large number of tests in this group is due to the fact that these analyses were performed first in case the amount of serum should prove scanty; furthermore, owing to the character of relative percentage figures, no material was excluded.

necessary, as the samples were from the start separated into two portions. Immediately before chemical analysis the samples were thawed, and divided into 0.6 ml portions in alkali-free glass tubes numbered I-V. One such tube was used for one or more of the assays shown below. Since many of the specimens were too small for all the originally intended tests, an order of priority was set up from the start, as follows.

A. *Serological analysis.*

B. *Biochemical analysis.*

Total protein, paper electrophoresis.

Phosphorus, alkaline phosphatase.

Calcium.

Sodium, potassium.

This system naturally resulted in a greater number of analyses in the first groups than in the last. This is seen from

Table 35. As is apparent, there is also some wastage owing to the discarding of a few figures that were either unreasonably high or unreasonably low. Too-low values may probably be explained by pipetting off the first specimen (for serological investigation) from an incompletely thawed sample, resulting in too-low concentration of all the solids in the remainder of the sample. Too-high values were noted in a few cases in which the samples were small. Experiments have shown that, under such circumstances, slow distillation of water from the specimen to the walls of the tube and the undersurface of the stopper may account for an increase in concentration of 10% or more. The number of analyses discarded for one of the above reasons is small in relation to the wastage due to insufficiency of serum. The question arises

TABLE 36. *Chemical analysis of the blood. I. Total serum protein g%, and relative percentage of electrophoretic protein fractions. Means, 95 % confidence intervals, and significance.*

	Feeding groups				Standard deviation of whole series	Mean (difference IV-I)
	I	II	III	IV		
<i>Maternal blood</i>						
Total protein	6.62 ± 0.16	6.71 ± 0.20	6.65 ± 0.24	6.74 ± 0.22	0.74	0.12
Albumin	44.5 ± 1.12	44.9 ± 1.66	45.3 ± 1.54	44.7 ± 1.64	5.76	0.19
Globulins						
$\alpha_1$	7.5 ± 0.30	7.6 ± 0.36	7.4 ± 0.30	7.4 ± 0.40	1.37	-0.07
$\alpha_2$	13.2 ± 0.44	13.2 ± 0.50	13.0 ± 0.54	12.8 ± 0.74	2.26	-0.43
$\beta$	17.4 ± 0.52	17.2 ± 0.62	17.5 ± 0.66	17.3 ± 0.68	2.49	-0.12
$\gamma$	17.5 ± 0.68	17.1 ± 0.96	17.1 ± 0.90	17.7 ± 0.86	3.35	0.20
<i>Umbilical-vein blood</i>						
Total protein	5.67 ± 0.16	5.90 ± 0.22	5.84 ± 0.24	5.83 ± 0.20	0.67	0.16
Albumin	59.7 ± 1.32	58.7 ± 1.78	58.2 ± 2.00	57.5 ± 1.68	6.41	-2.18*
Globulins						
$\alpha_1$	5.1 ± 0.30	4.9 ± 0.36	5.0 ± 0.40	5.1 ± 0.38	1.34	0.02
$\alpha_2$	7.5 ± 0.44	7.4 ± 0.52	7.7 ± 0.46	7.7 ± 0.48	1.90	0.20
$\beta$	8.4 ± 0.48	9.4 ± 1.20	9.2 ± 0.92	9.0 ± 0.78	3.01	0.56
$\gamma$	19.3 ± 0.60	20.2 ± 0.86	19.9 ± 0.84	20.4 ± 0.72	2.84	1.10*
<i>'7 ½ m' blood</i>						
Total protein	6.75 ± 0.12	6.89 ± 0.14	6.78 ± 0.16	6.67 ± 0.10	0.69	-0.08
Albumin	59.1 ± 1.06	60.0 ± 1.18	59.2 ± 1.34	61.0 ± 1.26	5.43	1.88*
Globulins						
$\alpha_1$	5.0 ± 0.18	5.0 ± 0.20	5.4 ± 0.24	5.2 ± 0.22	0.88	0.18
$\alpha_2$	13.4 ± 0.52	13.4 ± 0.64	13.5 ± 0.60	13.6 ± 0.62	2.61	0.19
$\beta$	11.3 ± 0.34	11.3 ± 0.32	11.1 ± 0.40	10.6 ± 0.44	1.71	-0.68*
$\gamma$	11.2 ± 0.56	10.7 ± 0.62	11.1 ± 0.70	9.8 ± 0.58	2.75	-1.40***
<i>'30 m' blood</i>						
Total protein	6.91 ± 0.14	7.08 ± 0.14	6.93 ± 0.20	6.87 ± 0.16	0.49	-0.04
Albumin	60.9 ± 1.32	59.9 ± 1.24	61.5 ± 1.62	60.9 ± 1.32	4.45	0.03
Globulins						
$\alpha_1$	5.1 ± 0.22	5.3 ± 0.24	5.2 ± 0.36	5.2 ± 0.36	0.85	0.11
$\alpha_2$	11.4 ± 0.50	11.7 ± 0.44	11.9 ± 0.80	10.6 ± 0.54	1.84	-0.85*
$\beta$	11.1 ± 0.34	11.2 ± 0.30	11.0 ± 0.46	11.3 ± 0.54	1.28	0.12
$\gamma$	12.1 ± 0.70	11.9 ± 0.74	10.6 ± 0.94	11.8 ± 0.70	2.52	-0.25

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

For number of samples analysed, see table 35.

The confidence intervals are given in Table 39.

of whether these factors may also have influenced the results of the remaining material, though not enough to produce obviously unreasonable discrepancies. This

possibility cannot be excluded for certain, but the fact, as we shall see, that the mean values given in Tables 36 and 37 tally closely with the figures reported by earlier

investigators, and with a control series of 25 samples of cord and maternal blood,<sup>1</sup> suggests that it cannot in any case have been of major importance. It should also be stressed that the selection was made without knowledge of the feeding groups to which the various samples belonged. Control calculations, in which parts of the discarded material was included and employed for establishing quotients with sodium as reference, showed in no case any further significant differences between the feeding groups at '7½ months'. In the case of the electrophoretic findings, which are only represented by relative figures, the circumstances described are irrelevant.

#### Total protein

*Methods.* The total nitrogen was estimated on 0.10 ml of serum by a standard micro-Kjeldahl method. A mixture of  $\text{CuSO}_4$  and  $\text{K}_2\text{SO}_4$  was used as catalyst. The total protein for each sample was obtained by multiplying the total nitrogen by the factor 6.25. This factor was used in order to render possible comparisons with earlier investigations. It is lower than that given by Cohn et al. (1946), but in this investigation no correction was made for non-protein nitrogen.

The total mean for the maternal blood is 6.67 g %, which tallies fairly well with the figures published by Oberman, Gregory, Burke, Ross, and Rice (1956) and by Sternberg, Dagenais-Perusse, and Dreyfuss (1956), but is slightly above those reported by Bouman (1956).

The total mean for the umbilical-vein blood is 5.80 g %, which again is in accordance with earlier reports (Pfau, 1954; Beckman, 1955; Sternberg et al. 1956); Oberman's figures (1956) are rather higher (6.11 g %), and derive from a series of 26

cases. The results of the '7½ months' examination vary between 6.67 and 6.89 g % (Oberman et al. reported a mean of 6.68 g % for the age-group 7-11 months). The '30 months' values are slightly higher, at 6.87-7.08 g %.

A comparison of the values for total protein in the various feeding groups shows no significant differences, either at '7½' or '30 months'.

#### Protein fractions

*Methods.* Paper electrophoresis was carried out in an apparatus described by Goa (1955) at 300 V for six hours in a veronal buffer, pH 8.6,  $\mu = 0.05$ . The amount of serum used was 0.01 ml, and this was applied to a Whatman No. 1,  $4 \times 40$  cm paper in all runs. The protein components were dyed with 1 % bromphenol blue dissolved in 96 % ethanol, saturated with  $\text{HgCl}_2$ . The excess dye was removed by 0.5 % acetic acid as described by Kunkel and Tiselius (1951). The resulting coloured zones were cut out and the dye eluted with 0.2 N NaOH in 96 % ethanol ( $v/v = 1/1$ ) for two hours. The extinction of the eluates was finally read at 595 m $\mu$  in a Beckman spectrophotometer model B, using 1 cm cuvettes.

The results, expressed per cent of the total protein, are collected in Table 36.

#### Albumin.

The total mean for the maternal blood was 44.8 %. This figure tallies with that given by Sternberg et al. (1956), but is rather higher than that reported by Oberman et al. (1956). Bouman (1956), on the other hand, found a much higher mean (58.8 %).

The mean value for umbilical-vein blood was 58.6 %. Beckman reported a value of 58.18 % for his series, and Oberman et al. 50.27 %. The means for the '7½ months'

<sup>1</sup> To be published.

examinations varied between 59.1% and 61.0%, and the '30 months' value between 59.9% and 61.5%, among the various feeding groups. The difference at '7½ months' between group IV and group I is of only probable significance (\*).

#### $\alpha_1$ -globulin.

The total mean for the maternal blood was 7.5%, and for the umbilical-vein blood 5.1%. The mean for the '7½ months' tests varied among the various feeding groups between 5.0 and 5.4%, and for the '30 months' tests between 5.1 and 5.3%. Widely diverging figures are to be found in the literature concerning the  $\alpha_1$ -globulin (Mancia, 1954; Drevon, Pigeud, and Donikian, 1955; Sternberg et al., 1956; Oberman et al., 1956). The results we have obtained accord fairly well with those published by Kropp (1950). The scatter for the entire series is less than seems to have been usual in earlier investigations. Concerning the  $\alpha_1$ -globulin, the differences that exist between the feeding groups are only very small, and in no cases are they significant.

#### $\alpha_2$ -globulin.

The total mean for the maternal blood was 13.1% and for the umbilical-vein blood 7.6%. The means for the '7½ months' tests varied between 13.4 and 13.6%, and for the '30 months' tests between 10.6 and 11.9%. The difference between the feeding groups IV and I at '30 months' is of only probable significance (\*).

#### $\beta$ -globulin.

The total mean for the maternal blood was 17.3%, and for the umbilical-vein blood 8.9%. The mean for the '7½ months' tests varied between 10.6 and 11.3%, and for the '30 months' test between 11.0 and 11.3%, among the feeding groups. As can be seen from the table, again there

are no highly significant differences between any of these.

#### $\gamma$ -globulin.

The total mean for the maternal blood was 17.4%, which tallies closely with figures reported in the literature (Oberman et al., 1956; Sternberg et al., 1956). The total mean for the umbilical-vein blood was 20.2% (Kropp gives a mean value of 20.5%). At '7½ months' the means varied between 9.8 and 11.2%, and at '30 months' between 10.6 and 12.1% among the different feeding groups. The figures obtained tally, on the whole, well with the results of Oberman et al. (1956), and others, and the difference between the  $\gamma$ -globulin level in the umbilical-vein blood and samples taken subsequently is of the same order of magnitude as reported in earlier series (Caspari, Negri, and Sticca, 1956; Pfau, 1954; Velasco, Lobo Parza, Yaues, and Gonzales, 1956; Oberman, 1956).

There is a highly significant (\*\*\*) difference between groups I and IV at '7½ months', group I showing higher values than group IV (the late-weaned infants).

#### Phosphorus and alkaline phosphatase

*Methods.* 'Inorganic' (acid-soluble) phosphate and alkaline phosphatase were determined on the same sample of serum. The phosphatase activity was assayed as the amount of phosphate liberated at the optimum pH, 10, during 15 minutes. A unit of phosphatase was defined as the amount of the enzyme required to set free 0.33 mg of phosphorus under the conditions of the test.<sup>1</sup> The reagents and procedure described by King (1946) were employed for the assay of enzymatic activity. The phosphate was analysed by a modification of the phosphate method described by Magnusson and Sylvan

<sup>1</sup> Since 0.33 mg phosphorus corresponds to 1 mg phenol the unit is equivalent to that used by King and Armstrong (1934).

TABLE 37. Chemical analysis of the blood. II. Phosphorus, alkaline phosphatase, calcium, sodium, and potassium. Means, 95 % confidence intervals, and significance.

		Feeding groups				Standard deviation of whole series	Mean difference IV-I
		I	II	III	IV		
Phosphorus mg per 100 ml	M	3.56 ± 0.30	3.56 ± 0.44	3.57 ± 0.58	3.69 ± 0.38	0.92	0.13
	0	5.80 ± 0.30	6.02 ± 0.50	5.83 ± 0.44	5.50 ± 0.36	1.24	-0.30
	7½	6.22 ± 0.14	6.32 ± 0.20	6.18 ± 0.22	5.85 ± 0.16	0.77	-0.37***
Alkaline phosphatase units/ml	30	5.72 ± 0.54	6.28 ± 0.52	5.72 ± 0.60	6.53 ± 0.56	1.50	0.81*
	M	18.25 ± 1.66	16.00 ± 1.90	18.36 ± 1.90	17.54 ± 1.94	6.33	-0.71
	0	10.98 ± 1.72	11.22 ± 2.00	11.26 ± 2.02	11.30 ± 1.60	6.08	0.32
Calcium mg per 100 ml	7½	18.22 ± 1.20	18.08 ± 1.20	18.25 ± 1.94	15.94 ± 1.26	5.64	-2.28**
	30	17.24 ± 1.20	16.00 ± 1.28	16.44 ± 2.68	17.76 ± 1.76	4.33	0.52
	M	9.62 ± 0.32	9.66 ± 0.40	9.62 ± 0.36	9.41 ± 0.34	1.17	-0.21
Sodium mEq/l	0	10.66 ± 0.50	10.54 ± 0.40	10.24 ± 0.60	10.24 ± 0.46	1.47	-0.42
	7½	10.19 ± 0.16	10.51 ± 0.24	10.37 ± 0.34	10.73 ± 0.28	0.95	0.54***
	M	137.06 ± 1.62	138.49 ± 2.56	138.21 ± 2.90	138.51 ± 2.46	6.84	1.45
Potassium mEq/l	0	141.46 ± 2.02	144.94 ± 2.46	142.00 ± 1.64	137.66 ± 2.10	6.38	-3.80**
	7½	140.41 ± 1.26	140.44 ± 1.52	138.67 ± 2.78	141.11 ± 1.88	5.57	0.70
	M	4.47 ± 0.18	4.43 ± 0.24	4.78 ± 0.48	4.64 ± 0.32	0.89	0.17
The confidence intervals are given in Table 39.	0	5.50 ± 0.38	6.00 ± 0.60	5.90 ± 0.60	5.44 ± 0.28	1.19	-0.06
	7½	4.96 ± 0.20	4.89 ± 0.16	4.72 ± 0.30	4.83 ± 0.18	0.67	-0.13

Feeding groups. I birth-2 weeks, II 1-2½ months, III 3-6 months, IV 6½ months or more of plain breast feeding.

M = Maternal blood, 0 = umbilical-vein blood, 7½ and 30 = blood obtained at '7½ m' and '30 m'.

For number of samples analysed, see table 35.

The confidence intervals are given in Table 39.

(1930). The control sample in this estimation also served for the determination of acid-soluble phosphorus.

All determinations were done in duplicate. The standard error of the method was calculated from the duplicates. The standard error of a single analysis was ±2.1% for acid-soluble phosphorus and ±2.9% for alkaline phosphatase.

The results can be seen from Table 37.

**Phosphorus.** The total mean for the maternal blood at delivery was 3.60 mg %. The figure for umbilical-vein blood is higher, at 5.78 mg %. Todd et al. (1939) give a value of 5.5 mg % for the umbilical-vein blood, and Graham, Barness, and

György (1953) report 5.7 mg % in the 'capillary' blood of new-born infants who had not yet been fed. At '7½ months' the total mean was 6.15 mg %, and at '30 months' 6.09 mg %.

Concerning the '7½ months' figures, there is a significant (\*\*\*) difference between the extreme groups, I and IV, and between groups II and IV. The bottle-fed infants thus have a higher serum phosphorus than the breast-fed, as Graham et al. (1953) demonstrated in infants during the first month of life. Concerning the '30 months' values, there are no highly significant differences between the groups.

The alkaline phosphatase values were

strikingly lower in the umbilical-vein blood than the maternal blood (11.2 and 17.6 units respectively, see Table 37). At '7½ months' and '30 months' the total means were 17.6 and 16.9 units respectively. At '7½ months' the figure for the plain-breast-fed group (IV) is significantly (\*\*) lower than that for the other groups (I, II, and III).

### Calcium

*Methods.* Calcium was determined on 0.2 ml samples of serum by direct titration against ethylenediaminetetraacetate (EDTA), using murexide as indicator (Schwarzenbach, Biedermann, and Bangert, 1946), as described by Lehmann (1953).

All determinations were done in duplicate. The standard error of the method was calculated from the duplicates. The standard deviation of a single analysis was found to be  $\pm 1.0\%$ .

The calcium values are given in Table 37. The total mean for the maternal blood was 9.58 mg %, as compared with 10.46 mg % in the umbilical-vein blood. The latter value is almost identical with that found in '7½ months' infants, 10.47 mg %. The figures seem to correspond closely to reports in the literature (cf. Stearns, 1939; Gyllenswärd and Josefsson, 1957). The difference between groups I and IV at '7½ months' is significant (\*\*\*), the infants reared at the breast showing the higher level. Similar findings have been published by Graham et al. (1953). Calcium estimations were not carried out at '30 months'.

### Potassium and sodium

*Methods.* The determinations were performed as follows. Serum was diluted 1:100 with redistilled water containing 0.03 % of a non-ionic wetting agent (Berol WMA-09).

A mixed stock standard containing 40 mEq/l of sodium and 4.2 mEq/l of potassium was diluted in the same manner. The serum samples were read against the diluted standard in a Beckman model DU spectrophotometer with a No. 9200 flame attachment. All determinations were made in duplicate. The standard error of the method was calculated from duplicate determinations of sodium and potassium. The standard deviation of a single analysis was  $\pm 1.8$  mEq/l for sodium, and  $\pm 0.16$  mEq/l for potassium.

As a control of the method, serum from 60 healthy adults (students and laboratory staff) was examined. The mean values and confidence intervals were, for sodium  $141.2 \pm 3.1$  mEq/l, and for potassium  $4.20 \pm 0.45$  mEq/l.

The results of the sodium and potassium determinations are shown in Table 37. The total mean sodium level for the maternal blood was 137.9 mEq/l, for the cord blood 140.9 mEq/l, and for infants aged '7½ months' 140.3 mEq/l. There are no differences between the feeding groups. The total mean potassium of the maternal blood, 4.56 mEq/l, was considerably lower than that of the umbilical-vein blood (5.66 mEq/l). The figure for infants aged '7½ months' was 4.88 mEq/l. Sodium and potassium estimations were not carried out at '30 months'.

There were no significant differences at '7½ months' between the different feeding groups.

### Discussion

With regard to total serum protein there was no significant difference between the groups. The gamma-globulin level was significantly higher in groups I, II, and III than in group IV. The reason for this is not clear. One explanation

tion would be that this difference reflects a difference in the general pattern of infection between the different groups (see discussion, p. 67). Another possibility might be that species-non-specific protein or other substances (e.g. milk bacteria) administered had acted as antigen.

There were no other differences of importance with regard to the electrophoretic fractions. The very slight difference in relative albumin content between groups I and IV can be regarded as secondary to the globulin difference.

The significantly higher calcium level in group IV compared with group I at '7½ months' indicates clearly that the serum calcium does not stand in direct relation to the calcium content of the diet. Concerning the inorganic phosphorus, on the other hand, the amount of this substance in the diet is reflected in the serum level: the groups that received some amount of cow's milk (groups I, II, and III) showed significantly higher levels than group IV. Similar experience has been reported by several teams. It has been maintained that the serum calcium may be inversely related to the serum phosphorus, and that the product calcium  $\times$  phosphorus is constant

even in children. A high intake of cow's milk would then result in a low serum calcium, and an increased propensity to develop tetany (Bakwin, 1937; Gardner, 1952). It is uncertain, however, whether this would apply in the case of moderate divergencies of the order of magnitude encountered in this connexion.

It is noteworthy that the infants that received cow's milk (groups I, II, and III) also showed significantly higher alkaline-phosphatase values than those reared solely at the breast (group IV). Thus there was a calcium-alkaline-phosphatase shift in the same direction as in rickets. The similarity is clearly only a superficial one, however, as the serum-phosphorus level was also raised, and X-ray examination of the bones in no case revealed signs of rickets.

There was no significant difference between the feeding groups with regard to other components.

The results presented represent a very considerable normal series for certain childhood age groups. Owing to the circumstances discussed on p. 75, we do not wish to stress this, but have used the figures only for purposes of comparison.

## 5. Appendix, A Comparison Between Artificial Mixtures Prepared from Fresh Milk and Dried Milk

During recent years commercial dried-milk products have come more and more into use in infant-feeding in Sweden. When planning the investigation now described, it was decided at the same time to collect enough material to compare the results of artificial feeding with mixtures prepared

from fresh and dried milk. No special measures were required for this purpose. In the township of Kiruna the supply of fresh milk of really high quality is at times limited, and a commercial dried-milk preparation for infant-feeding is a boon of which advantage is often taken.

TABLE 38. *Comparison between fresh milk and dried milk preparation. Means, feeding group I.*

The prefix - indicates that the values for dried milk were the higher.

Time		Fresh milk <i>n</i> = 83 <sup>1</sup>	Dried milk <i>n</i> = 60 <sup>1</sup>	Difference fresh milk - dried milk
'3 m'	Sedimentation rate mm/1 hr	10.5	9.4	1.1
	Haemoglobin, %	81	81	0
'7½ m'	Weight gain, kg	5.75	5.20	0.55**
	Ossification centres	13.3	14.0	-0.7
	Height, cm	69.8	70.3	-0.5
	Sedimentation rate mm/1 hr	11.2	11.3	-0.1
	Calcium, mg %	10.2	10.1	0.1
	Phosphorus, mg %	6.2	6.2	0
	Alkaline phosphatase, units/ml	17.0	20.2	-3.2**
	α <sub>2</sub> -globulin, %	13.3	13.7	-0.4
	β-globulin, %	11.2	11.3	-0.1
	γ-globulin, %	11.4	11.0	0.4
3-7½ m (β-period)	Rhinitis	1.01	1.15	-0.14
	Cough	0.81	0.90	-0.09
	Otitis media	0.10	0.05	0.05
	Upper resp. inf. with pyrexia	0.39	0.25	0.14
	Acute diarrhoea	0.41	0.15	0.26*

Feeding group I. Plain breast feeding birth-2 weeks.

<sup>1</sup> Total number of infants. Some data do not embrace every individual.

In the country district of Töre-Râneå, the supply of fresh milk is good, and it is natural that such milk is used to a great extent in artificial rearing of infants.

The composition of the artificial mixture employed is described in Chapter V. It can be seen that a 1:1 milk-and-water type of mixture was used in the case of both fresh- and dried-milk feeds.

The fresh-milk and dried-milk series are compared with regard to the same points as those taken up in the comparison of breast feeding and artificial feeding (section 1-4 of this chapter). Group I only is employed. Any difference ought reasonably to appear most plainly within this group, since the infants had been fed by the bottle from very early age.

The comparison was made between the results of examinations carried out at '3 m' and '7½ m'. Concerning the frequency of infection, term β (3-7½ months) only was employed, since in the present connexion it may be considered to be the most illuminating (Fig. 8, p. 63).

The result of the comparison is seen from Table 38. The weight-gain at '7½ months' showed a significant (\*\*) difference, the 'fresh-milk' values being the higher. The most obvious explanation would be that these infants must for some reason have consumed larger quantities of milk. In any case it cannot be warranted to conclude on the basis of these simple findings that a difference of biological significance exists between the two groups.

There was also a significant (\*\*) difference with regard to the serum-alkaline-phosphatase values. Even though the difference is slight, the slightly higher value for dried milk is surprising, since at the time of the tests this was enriched with a small quantity of vitamin D.

The higher mean for acute diarrhoea in the fresh-milk group is intriguing but since the significance of the difference between the groups does not exceed the 5 % level, it is probably best to refrain from speculations.

*Discussion.* In the manufacture of dried-milk products constantly improving methods are being adopted, and the risk of

structural changes that may adversely affect the nutritional value of the food is being cut down. The possible differences between dried-milk and fresh-milk mixtures are reduced before consumption partly because the latter are usually prepared from pasteurized milk, and partly because the milk is in any case usually brought to the boil during the preparation of the feed.

The findings summarized support the contention, daily borne out in practice, that there is no great difference between the results of feeding with fresh- and dried-milk mixtures, if the latter are prepared according to modern concepts.

## CHAPTER VIII

### GENERAL DISCUSSION

Health is 'a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity'. — Definition in the preamble to the Constitution of the World Health Organization.

#### General Views on the Concept of Optimum Nutrition

The criteria of optimum nutrition are still vague in many respects. Even in the absence of frank illness, function may be sub-optimum, and no clear limits can be defined. Furthermore, wide individual variations conditioned by genetic factors must be taken into consideration: what in one individual is optimum may be distinctly sub-optimum in another. These individual variations are even more apparent in growing subjects than in adults, since irregularities in the phases of development may magnify the individual variations, which are independent of these phases.

Much attention has been paid, in the work here described, to carrying out serological and chemical investigations in addition to purely clinical observations, with the object of elucidating the problem of the relationship between breast and artificial feeding. From the start it has been stressed that even these methods can reflect only roughly the true picture. It is known, for example, that considerable changes can take place in the composition of tissue which are not reflected in changed

composition of the blood. One of the most important new trends in nutritional research will undoubtedly be a multilateral approach to the problem based on quantitative determinations of not only the concentration of certain important substances in the blood at a given moment, but also the total blood and tissue content of these substances. In other words, the means of making a 'dynamic' approach may be expected to improve successively even in clinical research. The use of isotopes and immune-biological methods has already widened the path of approach.

The main object of the work now described has been to compare children reared at the breast and artificially, under conditions such that differences concerning factors other than the mode of feeding were kept to a minimum. Such differences can never be completely eliminated. In many investigations, however, especially in the past, hygienic conditions, for example, were such that their effect greatly exceeded any that can have been due to purely nutritional differences.

In many parts of the world the incidence

of breast feeding is tending to fall, and the same is true of Sweden. But the situation in this country is still good enough to make possible a study of the present kind without any difficulties. The figures for plain breast-feeding in the two research districts at 2 months varied between 45 % and 55 %, at 4 months between 28 % and 40 %, and at 6 months between 20 % and 30 %.

In this investigation, a considerable series was employed to obtain reliable average figures and to detect any differences between groups. For some of the tests only small series were available, as has been the case in many previous studies. Conclusions can be drawn from these only with great caution. On the other hand, it is important to stress that large series may conceal a few individuals with some widely divergent mode of reaction that is not reflected in the usual mean and scatter determinations. This state of affairs is of indisputable importance in connexion with the problems of our investigation. In isolated instances a child will show marked hypersensitivity to cow's milk (cow's-milk idiosyncrasy) or to ingredients in commonly-used feeds (for example, gluten factor in coeliac disease). It is also possible, though hardly proved, that cow's milk during the earliest period of life may exercise some antigenic effect that at a later stage may be unfavourable and that does not take place in children fed at the breast. On the other hand, breast milk alone, owing to its relatively low content of protein and mineral substances, may be inadequate if the requirements of these factors are for some reason particularly high as in premature infants.

In assessing results of nutrition it is essential to bear in mind that there is a

constant interplay between the nutritional factor and a variety of other environmental factors that may be operating at the time or that might at some earlier date have exercised significant influence. Thus, in this case, the question arises of influence upon the fetus of the mother's health during the pregnancy. Many comprehensive investigations have been designed to elucidate this problem, but the results have been conflicting (Ebbs, Tisdall, and Scott, 1941; Burke, Beal, Kirkwood, and Stuart, 1943; Sontag and Wines, 1947; Smith, C. A., 1949; Toverud, Stearns, and Macy, 1950; Moyer, Kelly, Macy, Mack, DiLoreto, and Pratt, 1954; McGanity, Bridgforth, and Darby, 1958). Critically viewed, however, they suggest that sub-clinical or even mild manifest deficiency states in the mother do not generally affect unfavourably the health of the infant. If this impression is correct, there is little reason to suppose that the children that took part in our investigation have suffered in this respect before birth, and in any case the inquiry we have carried out into the health of the mothers and into the dietary habits of these families has provided no evidence to indicate that any such difference exists between the groups.

Our study embraces only the period from birth to about  $2\frac{1}{2}$  years of age. This is a brief spell in a human life. It cannot, of course, be excluded that late effects of the considerable, important difference in mode of feeding during infancy may subsequently appear, but so far no evidence for this has been presented in the literature. Available data would on the contrary suggest that the organism has an exceptional capacity for equating the re-

sults of former nutritional deficiencies. An example of this is the apparently complete restitution of certain grave dystrophic states, including typical kwashiorkor, within a comparatively short time. And it has long been known that the skeletal changes associated with rickets tend to recover to a remarkable extent.

The artificial feeding used in the investigation now described was the simplest possible. It is conceivable that other mixtures, for example with lower mineral content, might eliminate some of the differences observed between the bottle-fed children and those fed breast milk alone. One fundamental feature cannot be modified, however, and that is the type of protein—a correction of the quantity cannot be complemented with a correction concerning the considerable qualitative difference.

In the investigation now described a comparison is made between children reared at the breast and children fed on a cow's-milk mixture from the bottle. Strictly speaking, an attempt ought to be made to refine the purely nutritional aspect of the problem by comparing two groups, both bottle-fed, the one with breast milk and the other with a cow's-milk mixture. For practical reasons such investigations are troublesome, especially if it is desired to use fresh breast milk, and have only been carried out in very limited forms (von Sydow and Faxén, 1954). At the same time it cannot be excluded that the occurrence of breast feeding implies a better general standard of care than artificial feeding. There is reason to believe that this would be reflected mainly at a psychological level, in connexion with the establishment of emotional rapport, however,

and possibly also in the time of development of various accomplishments.

One weakness in a comparative investigation such as described here is that no data are available concerning the quantity of milk consumed by the breast-fed infants. It is a known fact that the consumption of breast milk varies greatly, even among perfectly healthy infants of similar weight (Wallgren, 1944). In theory it would be possible to trace continuously the consumption of breast milk, but this is quite impracticable in a field study. Since, however, the content of organic constituents of the milk from different mothers varies, the total calorie-intake need not necessarily vary according to the volume of milk consumed. The consumption of artificial feeds also varies far more than the directions and notes would give one reason to suppose, but may be assumed to be more constant than that of breast milk. These factors must be borne in mind in assessing the differences between both mean values and individual observations.

As has been discussed in Chapter II, the chemical composition of cow's milk differs in several fundamental respects from that of human milk, including the nature of the fat and its content of unsaturated fatty acids. Cow's milk contains 0.10% of these fatty acids, and human milk 0.35%. During the period of the investigation it has been demonstrated in several quarters that fat with a high content of unsaturated fatty acids tends to lower the blood lipids, which has given rise to speculations concerning the possibility of prophylaxis against atherosclerosis by dietary means. Recently published preliminary data indicate that the effect

named may exist even in infants (Pomeroy, Goalwin, and Slobody, 1958; Lindquist and Malmerona). Our investigation was planned and started before the connexion between dietary fat and lipid metabolism was known. No estimations of

blood lipids were done. Certain available data suggest that there is no marked difference between the serum lipids of breast-fed and bottle-fed infants (Rafstedt, 1955).

### Discussion of Some of the Observations Made

It has been mentioned previously that the series was classified into four feeding groups with regard solely to the period of plain breast feeding. It is clear that the specific effect of breast milk, if there be any such effect, will not cease because a small quantity of cow's milk is introduced into the diet: the period of supplementary feeding must also be significant. In order to admit this point of view, another classification was tentatively tried, allowance being made for the period of pure breast-feeding and for the period of supplementary feeding (cf. Fig. 6, p. 46).

The 4 groups obtained in this manner finally included 274 of the children, distributed as shown in the figure. The effect of this classification has been compared with the other, based on the period of plain breast-feeding, for every factor. There have been some minor differences, but the conclusions have not been affected on any major point. We are therefore not publishing the statistical analyses.

The assessment of the effect of different modes of rearing is primarily based on a comparison of the two extreme groups, I (early weaned from the breast) and IV (weaned late from the breast). It would seem reasonable to assume that any existing differences would be most pronounced between these two.

Since in an investigation of this nature a substantial part of the material will in any case be referable to an intermediate

position, it seemed correct to report these figures (groups II and III). They have also been included in the statistical analysis, but were used for calculations of differences in only a very limited number of instances. Some of the results are discussed in more detail in Chapter VII, to which the reader is referred.

With regard to *physical development* more rapid gain in weight and height was noted at '7½ months' in group I (weaned early from the breast) than in group IV (weaned late). There was a corresponding difference in the development of ossification centres: in the case of the girls this reached the 0.1 % level, and for the combined series 1 %. At '30 months' these differences were equated.

Expressed per cent, the difference between groups I and IV at '7½ months' is greatest with regard to weight. It would of course be of considerable interest to know how these weight differences are distributed with regard to adipose tissue and the various organs, but for obvious reasons no such information is available.

The differences in skeletal development are relatively small, but warrant attention nonetheless, since it is in general assumed that the growth of the bones is not particularly sensitive to moderate variations in the quantity and composition of the

diet. With regard to the reasons for the differences noted, the first points to be considered are undoubtedly the very great differences in the total intake of the main nutrients, notably proteins, calcium, and phosphorus (cf. Table 6, p. 35).

*Clinical examination* was carried out with great regularity throughout the first year of life. To illustrate the effectivity of the prophylactic measures, in not a single case could overt signs of deficiency disease be detected. *Rickets* is a constant threat in this part of the world. Although about 3 % of the infants showed raised serum-alkaline-phosphatase values, in no case was there X-ray evidence of this disease at either '7½' or '30 months'. Another indicator of the efficiency of the medical welfare care is the incidence of *anaemia*. In our series at '7½ months' there were only 5 infants out of 402 in whom the haemoglobin was lower than 70 % (10.3 g/100 ml). In all feeding groups the average level was 83–84 % (12.3 g/100 ml), which matches nicely the generally accepted normal value for the age.

One of the most important features of the investigation was the careful recording of the incidence of *acute infections*. The material was particularly suitable for this purpose. Apart from a difference with respect to the number of siblings of school age, with a higher value in group IV, a detailed inquiry revealed close similarity between the feeding groups with regard to all significant points that might influence the pattern of infection. Many earlier investigations have been open to serious criticism on this score.

The number of cases of *named infectious illness* (measles, varicella, rubella, and exanthema subitum) was relatively limited

during the first year, as was anticipated. Altogether 66 cases of this type of illness were noted in a total of 63 infants. There was no tendency to overrepresentation in any group or groups.

The *acute infections of the upper respiratory passages and gastro-intestinal tract* were arranged in the following 5 groups: rhinitis, cough, otitis media, upper respiratory infection with pyrexia, and acute diarrhoea. With regard to cough and otitis media, higher values were noted in groups I+II (early-weaned) than in group IV (late-weaned). The significance of these differences does not exceed the 5 % level. A calculation of the incidence of different types of infection (cf Table 30, p. 66) revealed a difference at the 1 % level between groups I and IV, the values for the early-weaned infants being the higher. In evaluating these observations, the possibility of a greater risk of infections contracted by siblings at school among the infants of group IV should be borne in mind.

At 3 months the figures for *sedimentation rate* differed significantly, rising successively through the groups, from IV to I. At '7½ months' there was no such difference. The number of cases with *pyrexial incidents* was fairly small, and no difference between the groups was established. There were no differences with regard to the *antistreptolysin* and *antistaphylolysin* titres. The *gamma globulin* content of the serum was lower in group IV than in the other three groups, as we shall see.

*Dental examination.* The time of eruption of the milk teeth did not show any difference of importance between the various feeding groups. With regard to the

incidence of caries at '30 months', a difference of probable significance was observed, the values for group II (early-weaned) being higher than those of group IV (late weaned).

*Antibody response.* The post-vaccination titres of diphtheria, pertussis, and influenza B were measured. No difference could be established between the feeding groups, but in the case of influenza B there was too little material for statistical assessment.

*Biochemistry.* With regard to the total serum protein no differences were recorded. Of the electrophoretic fractions the only one showing a difference of high significance was the gamma-globulin, which at '7½ months' was lower in group IV than in the others. The serum calcium, phosphorus, and alkaline phosphatase also showed differences, the two last-named being significantly lower in group IV at '7½ months' than in the other groups, and the serum calcium at that time being significantly higher. At '30 months' there were no longer any differences.

The arguments presented have throughout been based upon a comparison between infants fed solely at the breast and infants weaned to the bottle at some date before the 'normal' completion of nursing. Primarily, the comparison has been between plain-breast-fed infants (group IV) and babies weaned from the breast within the first month of life (group I). In certain respects differences between the groups were established. These include physical development, and certain clinical and biochemical findings. It cannot be stated unequivocally which of the figures are the "normal" ones. For instance, it

cannot be established whether height-gain and development of ossification centres are accelerated in bottle-fed infants, or whether they are delayed in the breast-fed. Similar arguments may be put forward about the serum calcium, serum phosphorus, and serum-alkaline-phosphatase. With regard to certain other data the answer can more readily be given. If the incidence of infection is indeed lower with a certain type of rearing, this must be interpreted as evidence of the superiority of that type of rearing provided it has no essential disadvantages in other respects. In this investigation there has been a tendency towards lower incidence of acute upper respiratory infections among the infants reared solely at the breast for a long period (group IV) than among those weaned early from the breast (group I). The difference became significant only when a special classification was adopted, however, regard being paid to the average incidence of different types of infection. On comparison of the incidence of infections of a given type or of the total number of all types of infective incidents the differences were never more than probably significant.

The present study is by no means exhaustive, with regard to the assessment of breast feeding compared with cow's-milk feeding. We have endeavoured to achieve an objective appreciation of certain aspects of the problem, by using certain new conceptions and a combined clinical, biochemical, and serological approach. Many features are not touched upon. The principal of these is the psychological side of the problem. Nursing implies continuous, intimate contact between mother and child, which cannot be guaranteed in bottle

feeding even if the possibility of such rapport is not excluded. With regard to the somatic result of rearing, it has already been stressed that averages and figures for scatter cannot provide a complete picture. The large groups may contain individuals for whom a certain mode of rearing may be of much greater importance than would appear from the final, calculated averages. It is possible that such 'brittle subjects' may in isolated cases be totally concealed because unsuccessful attempts at weaning to the bottle resulted in continuation of breast feeding over a period longer than would otherwise have been the case. This may have occurred, but its importance in practice is probably small.

Important points have been omitted, and these include the lipid question and matters concerning the intestinal flora. With regard to the latter, the fundamental differences between breast- and bottle-fed infants have long been recognized, but practically nothing is known about their possible significance. About the lipids, it is clear that the content of unsaturated fatty acids is higher in human milk than in the usual cow's-milk mixtures, but again hardly anything is known about

whether this is of any biological importance, since the cholesterol-reducing effect of the unsaturated fatty acids reported in experiments on animals and man has been apparent only with fairly extreme variations in the diet.

It must be stressed that the investigation now presented is based upon a comparison between breast feeding and a certain defined type of artificial feeding. It is possible, and even probable, that some of the observations, for instance those concerning weight- and height-gain and development of ossification centres, might have been different if another cow's-milk mixture had been employed.

Finally, it is extremely important constantly to bear in mind the fact that throughout the investigation all the participating infants were kept under strict clinical observation. Under less favourable circumstances, particularly with regard to hygiene, certain of the findings might well have been different. This is exceedingly important with regard to the conditions prevailing in developing countries, where breast feeding may still spell life or death for the infant.

## SUMMARY

### *Previous investigations*

The monograph opens with a survey of the literature (Chapter I) comprising 20 pages. In a separate section on the comparative biochemistry of human and cow's milk (Chapter II) the usual chemical data are discussed and in addition the results given of certain recent studies.

### *Personal investigations*

The series consists of 402 infants. In those cases in which breast feeding could not be carried through to the full the diet was supplemented with a 1:1 mixture of cow's milk and water with 5 % of sugar and 1 % of wheat flour. Every child was frequently and regularly examined during the first year of life, and subsequently kept under observation in accordance with the standard child-welfare-centre pattern. Special investigations involving clinical examination, blood tests for chemical and serological estimations, and at certain ages X-ray of the bones and dental examination, were carried out at birth, '7  $\frac{1}{2}$  months' (90 % of the children were aged 6-9 months), and '30 months' (90 % aged 26-34 months). The timetable for the special investigations and for certain of the immunization-procedures is shown in Fig. 4, p. 41.

*Results.* The series was divided into 4 groups, according to the duration of breast feeding (see Fig. 6, p. 46). The confidence interval was systematically calculated between the extreme feeding groups, that is, between the infants weaned very early (group I) and very late (group IV) from the breast. The findings for the intermediate groups were also taken into account in the statistical assessment of the results, but only in part of the series were they utilized in estimating confidence intervals.

A survey of those results that led to the calculation of confidence intervals is to be found in Table 39, p. 92. These are commented upon in full in Chapter VII.

With regard to *physical development* (weight, height, ossification centres), the values were significantly higher at '7  $\frac{1}{2}$  months' for group I (early-weaned) than for group IV (late-weaned).

The frequency of certain *acute infections* (cough, otitis media) was higher in groups I and II (early-weaned) than in group IV (late-weaned), but the differences are moderate, and the degree of significance does not exceed the 5 % level. A comparison with regard to different types of infection shows a higher incidence (\*\*) in group I (early-weaned) than in group IV (late-weaned).

TABLE 39. Condensed figures for confidence intervals collected from the various tables of results.

The figures in the table represent the differences between group IV (late-weaned) and group I (early weaned). The prefix - indicates that the values for group I were the higher.

	Table	'3 months'	'7½ months'	'30 months'	0-12 months
<i>Clinical examination</i>					
Weight-gain, kg.	19	-0.03 ± 0.13 <sup>1</sup>	-0.62 ± 0.25***	-0.66 ± 0.51*	
Height, cm.	20		♂ -1.0 ± 0.85*	♂ -0.1 ± 1.4	
			♀ -1.4 ± 1.0**	♀ -0.9 ± 1.5	
Ossification centres	21		♂ -0.3 ± 0.7	♂ -0.7 ± 3.7	
			♀ -1.4 ± 1.1**	♀ -1.5 ± 4.5	
			♂ + ♀ -0.9 ± 0.7*		
Haemoglobin, %	23	2.0 ± 1.5*** <sup>1</sup>	0.0 ± 1.6	0.0 ± 1.9	
Sedimentation rate, mm/hour	25	-3.2 ± 1.5*** <sup>1</sup>	1.4 ± 2.3		
Acute infections of the upper respiratory tract and acute diarrhoea	28, 29				
Frequency of Rhinitis					-0.11 ± 0.22 <sup>2</sup>
Cough					-0.28 ± 0.22**
Otitis media					-0.05 ± 0.04**
Upper resp. infections w. pyrexia					-0.04 ± 0.13 <sup>2</sup>
Acute diarrhoea					-0.02 ± 0.11 <sup>2</sup>
Incidence of different types of infection	30				-0.36 ± 0.22**
<i>Dental examination</i>					
Time of eruption of teeth <sup>3</sup>	31				
Incidence of caries	33			A 0.78 ± 1.60 <sup>4</sup> B 0.33 ± 0.98 <sup>4</sup> B-A -1.29 ± 1.2** <sup>4</sup>	
<i>Antibody response to immunization</i>					
Diphtheria, units/ml	Fig. 10		3	3	
Pertussis, titres	Fig. 11		3	3	
<i>Biochemical analysis</i>					
Total protein, g%	36		-0.08 ± 0.16	-0.04 ± 0.21	
Electrophoretic values, %					
Albumin	36		1.9 ± 1.7*	0.0 ± 1.9	
α <sub>1</sub> -globulin	36		0.2 ± 0.4	0.1 ± 0.4	
α <sub>2</sub> -globulin	36		0.2 ± 0.7	-0.8 ± 0.7*	
β-globulin	36		-0.7 ± 0.6*	0.1 ± 0.6	
γ-globulin	36		-1.4 ± 0.8***	-0.2 ± 1.0	
Phosphorus, mg%	37		-0.37 ± 0.21***	0.81 ± 0.77*	
Alkaline phosphatase, units/ml	37		-2.3 ± 1.7**	0.5 ± 2.1	
Calcium, mg%	37		0.54 ± 0.32***		
Sodium, mEq/l	37		0.7 ± 2.3		
Potassium, mEq/l	37		-0.13 ± 0.27		

<sup>1</sup> Comparison of groups IV + III with group I.

<sup>2</sup> Comparison of group IV with groups I + II during the age period 3-7½ months, see Table 29.

<sup>3</sup> No difference between the different feeding groups.

<sup>4</sup> For explanation see p. 69.

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The incidence of caries at '30 months' is higher in group II (early-weaned) than in group IV (late-weaned), but the degree of significance does not exceed the 5 % level.

The antibody response to diphtheria and pertussis immunization shows no significant difference between any groups; and no significant difference is found with respect to influenza vaccination, but in this case there was very little material.

Concerning the serum biochemistry, there are notable differences at '7 ½ months' with regard to gamma-globulin, calcium, phosphorus, and alkaline phosphatase.

As an appendix a comparison is made between two sub-groups of group I, of which one had received a 1:1 feed prepared with *fresh milk* and the other a similar mixture made from a *dried-milk* preparation. A significant (\*\*) difference was revealed with respect to weight-gain and the serum level of alkaline phosphatase.

It should be stressed that the results presented refer to a certain defined type of artificial feeding, and furthermore that all the participating infants were kept under strict clinical observation.

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C. Holm

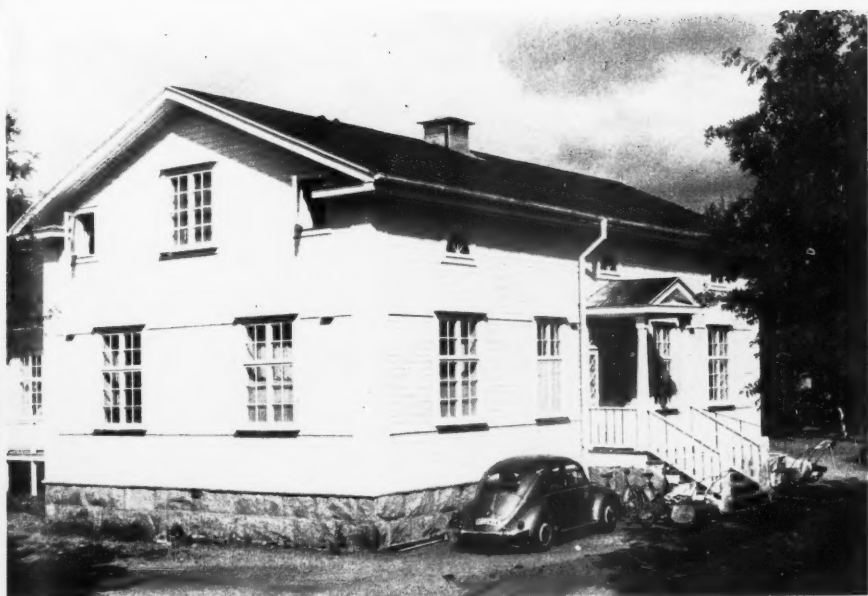
Kiruna in winter. In the background the heights of Kirunavaara, with iron-ore deposits.



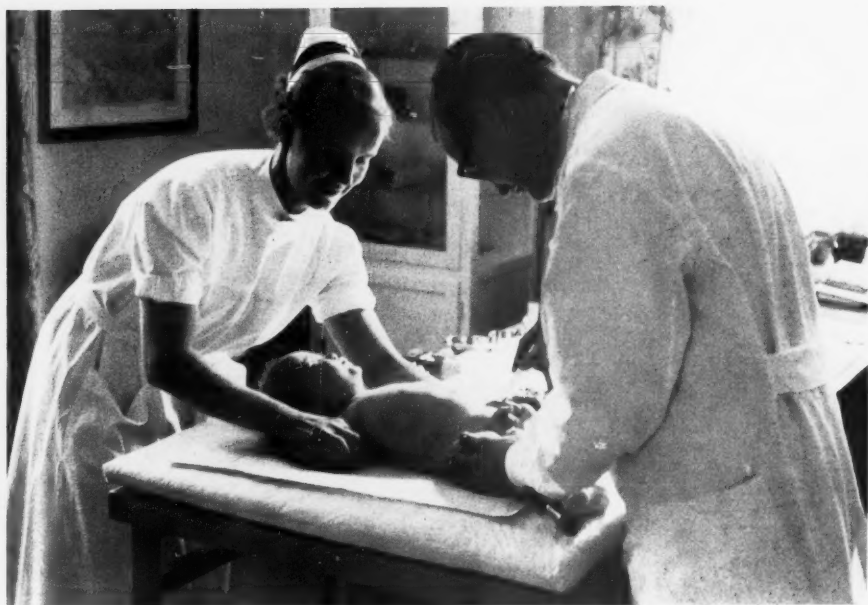
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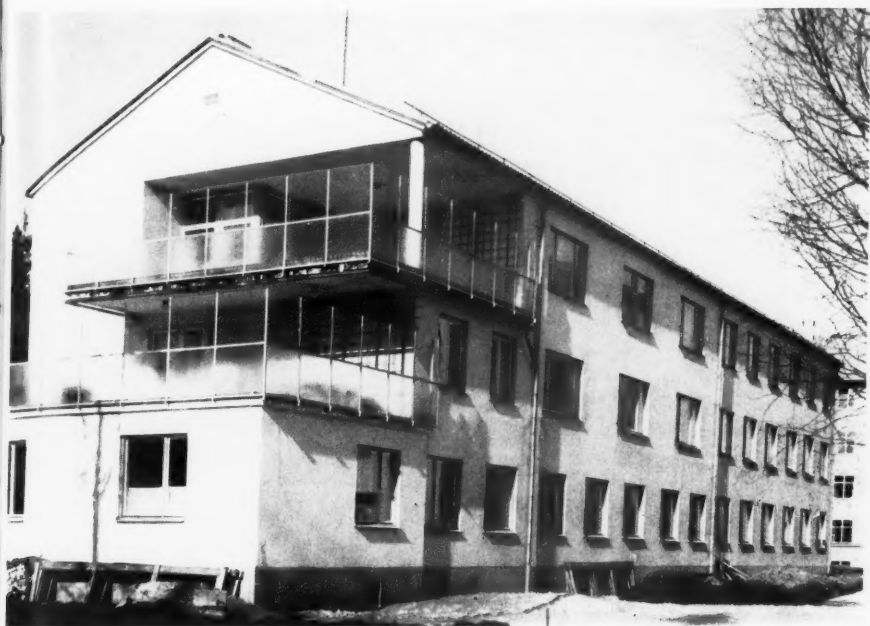
The Special Well-baby Clinic at work.



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Modern apartment houses in Kiruna.



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The Well-baby Clinic at Râneã.

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Typical farm in the Töre-Râneå district.

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Village Square, Töre.

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The Farmstead, the Forest, and the River — The Lowlands of Norrbotten.



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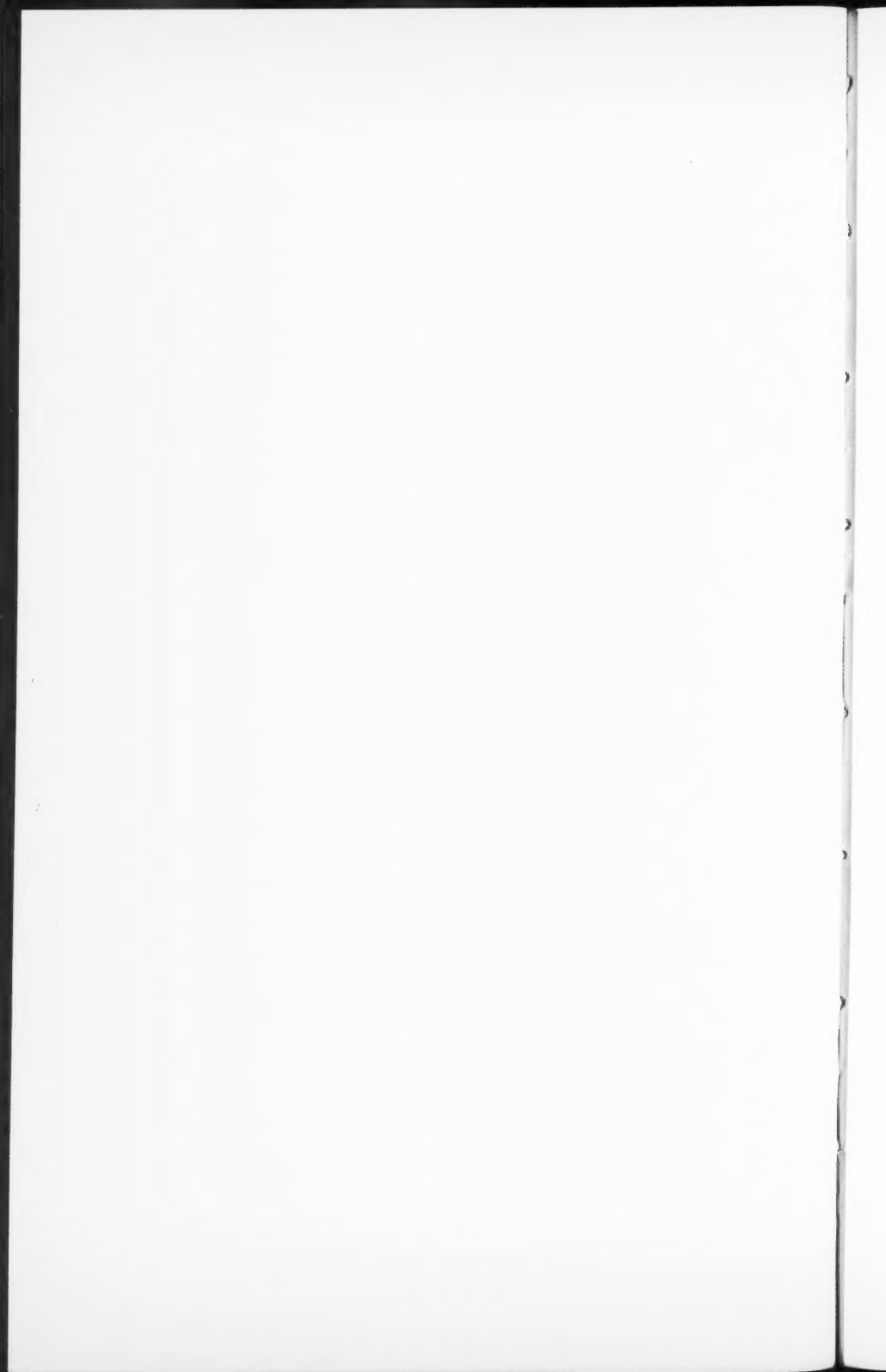
IN HONOUR OF

**BO VAHLQUIST**

ON HIS FIFTIETH BIRTHDAY

APRIL 11th, 1959

*Almqvist & Wiksells Boktryckeri AB · Uppsala*



IN HONOUR OF

# BO VAHLQUIST

ON HIS FIFTIETH BIRTHDAY

APRIL 11th, 1959

SPECIAL EDITOR, STIG SJÖLIN

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## BO VAHLQUIST, FIFTY YEARS

When Bo Vahlquist acceded by invitation to the professorship in pediatrics at Uppsala University in 1950 great things were expected of him. His earlier contributions to Swedish pediatrics and his scientific achievements were adequate grounds for such expectation. He has without doubt attained to the stature of a pioneer figure in European pediatrics and to a dominant position among Swedish pediatricians. He has made the Uppsala Children's Clinic a renowned center for research, to which pediatricians from near and far seek training and assistance in solving scientific problems.

Vahlquist is distinguished by clear-sightedness in dealing with actual problems, by meritorious research planning, and by carefully prepared experimental procedures. This is not the appropriate place to give an account of his numerous contributions. I will only briefly allude to his most important work. His first significant contribution concerned the iron content in the serum during normal and pathological childhood conditions. This investigation and a series of many-faceted hematological studies preoccupied him during the early nineteen-forties. Subsequent publications by his pupils and by himself disclose that he still entertains a predilection for the hematology of childhood. His hematological investigations have gained him recognition in wide scientific circles both within and without the borders of our country. Toward the end of the nineteen-forties Vahlquist began work on fresh research tasks and foremost among these were immunity problems in diphtheria. These studies led him to investigate the immunity attained by vaccination. For several years he has pleaded for a more extensive use of triple vaccination of infants in our Children's Welfare Centers, in order to produce a basal immunity against at least three infectious diseases, namely diphtheria, pertussis and tetanus. During the past five years, Vahlquist has devoted his investigative efforts also to other pediatric problems and to problems bordering on pediatrics. Foremost among these is a social hygienic study of infant nutrition. The latter, chiefly conducted as a field study in collaboration with a group of fellow-workers, is especially broadly planned and comprehensive, and necessitates a gigantic expenditure of time and efforts. At present we know only certain detailed results of this investigation. Hence it is with keen anticipation that we await the final results which will be published sometime this spring.

In more recent years Vahlquist has become deeply engaged in social medical work for handicapped (cerebral paretic) children. With purposefulness and energy he has steadily become a dynamic central figure in this heretofore neglected field. The opposition

launched against some of his proposed projects has but spurred him on to more painstaking efforts.

It is only natural that such an internationally recognized physician as Bo Vahlquist, possessed as he is of broad interests within his chosen field, should be called upon to solve international medical problems. Thus the World Health Organization has repeatedly engaged him to participate in various symposia and study groups. He has also served as Medical Consultant on educational questions and for the improvement of child care in certain southeast Asian countries.

Interested as Bo Vahlquist has been in all types of educational programs, he has fully succeeded in making the ordinary pediatric course for internes at Uppsala University a model of curricular perfection and content. Annually he invites pediatric physicians-in-chief to well-attended and highly esteemed instructional conferences in which current theoretical and practical problems are freely discussed and debated: a most unusual form of postgraduate specialist instruction indeed. He has written the excellent chapter on infectious diseases and the discussion of iron metabolism in Fanconi-Wallgren's *Lehrbuch der Pädiatrie*, and in collaboration with Rolf Zetterström the chapter on metabolic diseases in *Nordisk Lärobok i Pediatrik* (Scandinavian Textbook on Pediatrics).

The esteem and respect in which contemporary pediatricians hold Bo Vahlquist for his valuable contributions to the practical art of pediatrics and his scientific acquisitions in this field have been expressed in numerous ways. His kindness, courtesy and sense of humour in personal dealings, coupled with his common sense, reliability and sound judgment in professional affairs have made him both respected and loved by everybody who has had the privilege of knowing him. For over a year Vahlquist has served as chairman of The Swedish Pediatric Society. He has been honored by being commissioned as co-editor of several foreign scientific journals, and also of *Acta Pædiatrica*. In all these capacities Vahlquist has rendered conspicuous and highly creditable services. On this occasion the writer of these lines takes great pleasure, when Bo Vahlquist is being honored by former and present co-workers with this honorary volume of the *Acta*, in expressing his warm appreciation of valuable collaboration. I also make myself a spokesman for his older and younger co-workers as well as for his pediatrician friends in various countries in conveying their homage to our beloved fifty-year-old colleague.

Dear Bo, despite your increasingly extensive administrative and organizational tasks, may you find time and sufficient strength to advance toward your destined goal. Your star stands now in its zenith. Keep it in that position, at least during the initial decades of your next half-century!

Arvid Wallgren

## Treatment of Idiopathic Thrombocytopenic Purpura in Childhood

by GUNILLA BERGLUND and OVE BROBERGER

Idiopathic thrombocytopenic purpura (ITP) has been treated in many different ways, strongly suggesting that at present there is no ideal treatment. Splenectomy was introduced by Kaznelson in 1916. In many cases the operation had to be performed at a time when the patient had a tendency to severe bleeding and was thus combined with great risk of uncontrollable bleeding. Because of this a great advance was made when it became possible to reduce bleeding time to normal by platelet transfusions given immediately before operation. In 1951 Evans succeeded in proving the presence of platelet agglutinins in plasma from patients with ITP. Several years later there was further evidence of the occurrence of an antigen-antibody reaction in ITP, when Harrington *et al.* (1951) caused a marked thrombocytopenic purpura in a healthy person after giving a plasma transfusion from a patient with ITP. From a theoretical point of view, treatment with steroids ought to give a therapeutic effect in ITP by diminishing the antibody production. However, it has been found that the primary effect of such therapy is a decreased bleeding tendency and a reversion of bleeding time to normal and a subsequent increase in

the platelets, though far from all cases showed any such effect (Medical Research Council). According to Dameshek *et al.* (1958) it is important to give large doses for a long time. In their series where Prednison in a dose of 20 to 250 mg a day was given, the platelet count became normal in about 75 % of the cases after 50 days of treatment and absence from all bleeding manifestations could then be achieved by a smaller maintenance dose. Watson-Williams *et al.* (1958) reported remissions in 21 of their 42 patients treated with steroids, with permanent remission in cases having a short history, but more temporary remissions in patients with ITP of long duration. These results are also similar to those of Stefanini.

In children, however, it seems doubtful if such intense and long-lasting steroid therapy as recommended by Dameshek could be used. In an attempt to get some answer to this question we have compiled the cases from the Children's Clinic, Karolinska Sjukhuset.

### Methods

The platelets were counted by the method of Kristenson with a normal value of 200,000-350,000/cmm.

The bleeding time was estimated by Duke's method. Normal value 1-3 minutes.

The capillary fragility was examined with the tourniquet method with the pressure of the cuff between the systolic and diastolic pressure for 5 minutes.

The thrombocyte agglutination was performed as described by Stefanini & Dameshek (1955).

### Material

During the years 1951-1958, 28 children were treated, who fulfilled all the criteria of ITP as defined by Harrington *et al.* (1956). Nineteen were girls and 9 boys. The girls outnumbered the boys in all ages, though it is generally accepted that ITP before puberty is equally distributed among the sexes. This is in contrast to the finding that in later life the disease is 3-4 times more common in women than in men (de Gruchy, 1958).

Patients with thrombocytopenia of post-infectious origin as well as those cases associated with other hematological conditions were excluded.

All the patients had bleeding manifestations, prolonged bleeding time, increased capillary fragility and platelet counts below 35,000. The bone marrow was examined in 19 of the cases; in 2 a slight maturation arrest of the megacaryocytes was observed, while in 6 cases megacaryocytopoiesis was increased. In the remaining 11 cases the bone marrow smears were completely normal. In no cases were there any abnormalities in erythropoiesis or granulocytopoiesis. The spleen was not palpable in any case; this finding, however, does not preclude the possibility of some degree of splenomegaly, since the spleen may in some cases be pushed upwards. In the splenectomized cases the spleen was moderately enlarged in one third of the cases. In all cases the microscopical examination of the spleen revealed a normal appearance.

Thrombocyte agglutination was performed before any kind of therapy was given in 22 cases and in 9 of them agglutination occurred.

The series has not been divided into acute and chronic cases, because it was impossible to secure a sharp division between such

groups. However, 7 cases were mild with a fairly short history, 2 others were mild but long-lasting, and the remaining 19 had severe bleeding manifestations.

### Results

In all the mild cases neither steroid treatment or transfusions were given. In 7 of these cases the signs of ITP disappeared in a period ranging from 2 to 20 months (Fig. 1). Two cases still show a thrombocytopenia, though there is a complete absence of bleeding tendency. One of these (Y. A.) had during the first 38 months of illness a bleeding tendency which later disappeared completely so that during the last 4 years she has had normal menstruations in spite of a low thrombocyte count. Of all the mild cases she is the only one who has ever had a positive thrombocyte agglutination, and this still remains positive. Eighteen patients have been treated with steroids. These had either severe bleeding manifestations with need for acute treatment or the disease failed to show any signs of spontaneous healing. The drug most often used has been Prednisolon in a dose of about 1 mg/kg bodyweight and day given for at least 3 weeks and thereafter in successively diminishing amounts. Improvement has occurred in 6 cases, characterized at first by a decreased bleeding tendency and then a gradually increasing platelet count. In 12 cases no permanent effect was obtained, although a few cases showed transient improvement. Later 3 of these patients had a spontaneous remission, one completely, the other two with loss of the bleeding tendency, though the thrombocyte count still remains around 60,000.

Among the cases which have been

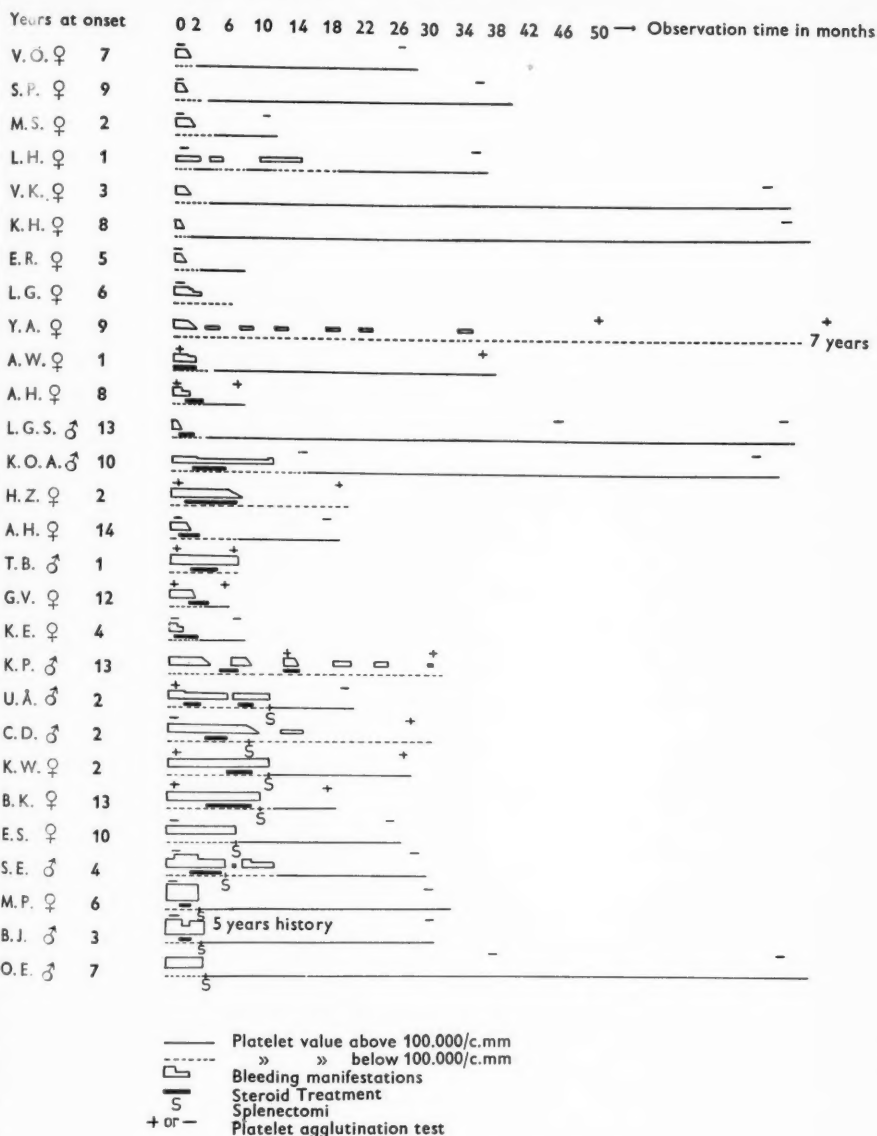


Fig. 1. Follow up diagram of the case histories.

treated with steroids without effect, 8 have also been splenectomized, and an additional patient (T. B.) will be operated in the near future. Five of the patients operated upon responded immediately with an increase of the platelets and a return of bleeding time to normal. They have since then had normal hematological values, the period of observation ranging from 8 to 30 months. In 2 other operated patients the bleeding tendency has disappeared, and in a third case after 2 months the thrombocyte count has also returned to normal. Whether this was an effect of the splenectomy may be doubtful. One patient did not improve after splenectomy, but remarkably enough responded well to steroid treatment later, though he did not show any benefit of this therapy before the operation. He has been free from symptoms for 18 months.

One further patient who was not treated preoperatively with steroids was splenectomized with good result.

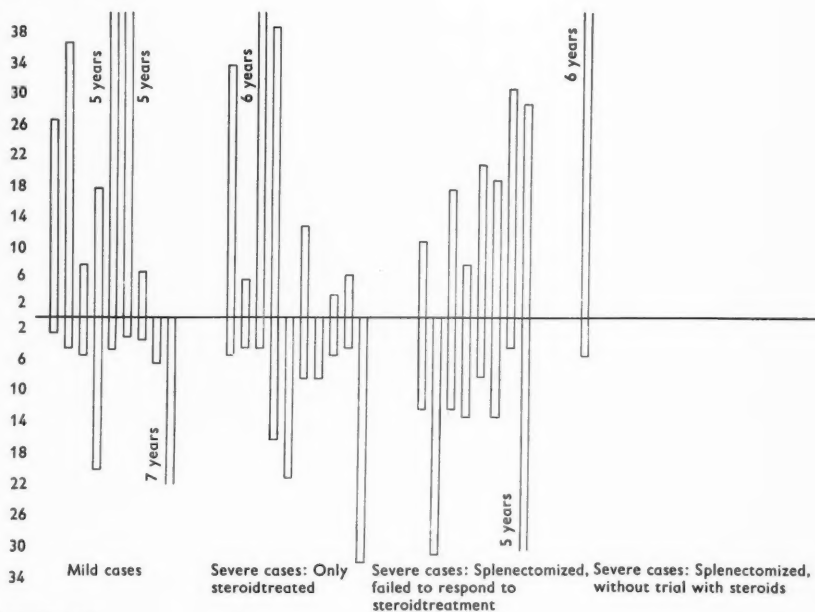
In the mild cases who have healed spontaneously the thrombocyte agglutination was negative in all cases except the long-lasting case, Y. A., which has been referred to earlier. Of the more severe cases some of which had responded to steroid treatment and some of which had to be splenectomized, the agglutination test has been positive in half of the cases. A positive thrombocyte agglutination should thus make one suspicious that one is dealing with a more severe form of ITP, but no information on the long-term prognosis can be derived at this time. Repeated agglutination tests have shown that these often become negative when the signs of disease disappear, but remain positive in many clinically symptom-free

patients with low platelet counts. In one splenectomized patient (C. D.), who is clinically symptom-free but with persisting thrombocytopenia, the thrombocyte agglutination test has reverted from negative to positive. The cause of this is obscure because he received platelet transfusions at the time of operation, and it is known that platelet transfusions may give rise to production of platelet antibodies. This is the reason why platelet transfusions have not been given more generally. Usually, as pointed out by Stefanini, a good result is obtained only after the first transfusions. Therefore the platelet transfusions have been restricted for use only in cases of severe bleedings during operations; other bleedings have been treated with transfusions of fresh whole blood.

### Discussion

From Fig. 2 it is seen that the effect of the steroid treatment is by no means as favorable in our material as in that of Dameshek *et al.*, but in our material we did not use such large doses as in some of their cases. Only one third of the steroid-treated patients recovered. Nor is there any evidence that the patients not responding to steroid treatment should not respond to splenectomy. The result of splenectomy has been excellent in 5 of 8 cases. This is about the same figure as found in adults where normal platelet values and normal bleeding time is found in about 50-80% after splenectomy (Conley *et al.*, 1956). The duration of the observation in our material has not, except in a few cases, been more than a couple of years, and during this time no signs of relapse have occurred, as described among adults. This

Number months of  
observation after  
complete remission



Number months with  
thrombocytopenia

Fig. 2. Observation period before and after remission with various therapy.

gives more reason for optimism regarding the prognosis in children than in adults. According to Dameshek & Reeves (1956) ITP may sometimes be only a part of the symptomatology of lupus erythematosus disseminatus and splenectomy will only accelerate the other manifestations of the disease. Examinations for "LE" cells have been performed in most cases during the disease before the steroid treatment, and in all cases in the follow-up investigations, but no such cells could be found. The serum electrophoresis has been normal in all cases and there have not been any of the other manifestations characteristic of lupus erythematosus.

Stefanini, among others, has called attention to the great risk of relapses especially after splenectomy. In none of our cases showing apparent recovery with a normal bleeding time and platelet count has any relapse occurred. One of the splenectomized patients (U. Å.) has been very susceptible to infections, and has even suffered a bacterial meningitis, from which he recovered with generally accepted treatment. The others, according to their parents, have been more resistant to infections after the operation than before.

In conclusion, ITP in children can be a relatively mild disease, which heals spontaneously, but more commonly it persists

as a severe and long-lasting disease requiring intensive treatment. In such cases steroids should be tried, because in addition to those who recover, including normalizing of the platelets, there may be some who have a remission with disappearance of the bleeding tendency making them more suitable candidates for splenectomy. In cases requiring operation, the most favorable time, if operation can be delayed, is some months after a period of steroid treatment.

In  $\frac{5}{8}$  of the cases the disease has disappeared completely after splenectomy, and in the remaining cases some improvement has occurred. No late complications or relapses have developed in this group of children. In adults a mortality of 8-10% is found, usually related to cerebral hemorrhages. In the present material there was no mortality and no case showed any symptoms of cerebral vascular insults.

### Summary

A series of 28 children with ITP treated during 1951-1958 is presented. Some mild cases required no treatment in any form, but most of the cases were treated with steroids, to which some responded with complete remission. The patients not responding to steroid therapy underwent splenectomy with a good result, except in one who responded to a renewed steroid therapy. No relapses, complications or deaths occurred in this group of patients.

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## An Autoradiographic Study of the Fate of Transfused Thymus Lymphocytes<sup>1</sup>

by H. DIDERHOLM and K. E. FICHTELIUS

The first attempt to trace transfused thymus lymphocytes within the recipient animal was published by Osogoe & Hitachi (1950). A suspension of cellular constituents from the thymus of rabbits was administered in a single injection into the ear vein of another healthy rabbit. Three days after the injection they found a striking focal accumulation of lymphocytes in the radicles of the interlobular portal vein, in the periportal spaces of the liver, and in the perifollicular regions of the spleen. When the lymphocytes were injured before injection no accumulations of lymphocytes were produced anywhere.

If radio-active phosphorus ( $P^{32}$ ) is injected into an animal, it will be incorporated into the desoxyribonucleic acid (DNA) of newly-formed lymphocytes, where it will remain as DNAP until the cells are broken down (Ottesen, 1948). In this way it is possible to tag young lymphocytes for transfusion into another animal. If thymus cells tagged with  $P^{32}$  are transfused from rats to another rat of the same litter, the cells can be recovered from the liver and spleen 24 hours later (Fichtelius, 1953).

The accumulation of tagged thymus cells in the spleen is still more pronounced

if the recipient has aseptic peritonitis (Fichtelius, 1953 and 1957a). In the rats with peritonitis a slight non-specific secondary response can be expected (Freund, 1953) with increased production of all antibodies normally produced by the recipient, and with proliferative changes in the spleen. Very important among these is plasma cell proliferation. This mainly occurs perifollicularly (Huebschmann, 1913; Björneboe & Gormsen, 1943; Fagraeus, 1948). It is in this particular region that Osogoe & Hitachi recovered the transfused, unlabelled thymus lymphocytes.

Basing his arguments principally on these results Fichtelius (1953) advanced a hypothesis about thymo-splenic integration in antibody formation: the thymus produces lymphocytes and the spleen forms antibodies with their aid. It is supposed that the thymus lymphocytes are transformed into plasma cells in the spleen. This hypothesis receives further support in a later paper (Fichtelius, 1957 b).

Osogoe's & Hitachi's results are in good agreement with Fichtelius' and the two complement each other. The weak point of the formers' method is that the

<sup>1</sup> This work has been supported by grants from The Swedish Medical Research Council.

cells are not labelled at all, and that the lymphocytes are shown in histological sections without the possibility of a certain, quantitative comparison. Fichtelius' cells are labelled and the method permits a better quantitative assessment but yields no information regarding the position of the transfused cells within the affected organs. An autoradiographic study of the fate of the transfused cells forms a natural complement to the investigations already carried out. The most important aim of such an investigation is to find out whether the transfused, labelled thymus cells can be recovered in the perifollicular regions of the spleen. At the same time one can try to confirm the results gained from previous transfusions of labelled thymus cells.

### Methods

Four-week-old male guinea-pigs weighing 170–200 g were used. A suspension of

lymphocytes in saline was prepared from the thymuses of five donors, labelled 24 hours earlier with  $3 \mu\text{CP}^{32}$  per g as orthophosphate. The suspension was injected into the saphenous vein of the recipient, which was killed after a further 48 hours. Pieces of spleen, liver and bone marrow, one lymph node at porta hepatis and one mesenteric lymph node were fixed in formalin and embedded in paraffin. 10 and  $20 \mu$  sections were prepared and placed on metacrylate slides and covered with Gevaert Dentsu Rapid film. The films were exposed for 28 days and developed in Kodak D 19b for 5 minutes. The method is described in detail by Odeblad (1952).

In all, three such transfusions were performed. The first recipient got  $2.5 \times 10^8$  cells in 0.6 cc suspension, the second  $5.1 \times 10^8$  cells in 0.8 cc, the third  $7.9 \times 10^8$  cells in 1.0 cc. The following control experiments were made. Two animals received  $1 \mu\text{C P}^{32}$  per g as orthophosphate subcutaneously. A third control got a suspension of  $10 \times 10^8$  thymus lymphocytes in 1.0 cc from five donors. The cells were heated to  $100^\circ\text{C}$  before injection into the saphenous vein. The con-



Fig. 1. Autoradiograph of spleen after transfusion of labelled thymus cells.



Fig. 2. Ordinary section of the same spleen as in fig. 1.



Fig. 3. Autoradiograph of spleen after injection of inorganic  $P^{32}$ .

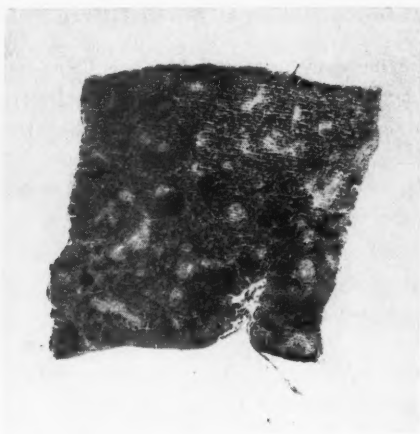


Fig. 4. Ordinary section of the same spleen as in fig. 3.

controls were killed 48 hours after the injection and handled in the same way as the recipients above.

### Results

The autoradiographs of the spleens of the three recipients that got living thymus lymphocytes showed a strong blackening especially in the case of the animal that had received most lymphocytes. The blackening was localized to the red pulp of the spleen while the white pulp showed no activity whatever. The blackening was throughout most marked perifollicularly (see Figs. 1 and 2). Fig. 3 shows the autoradiograph of the spleen of an animal that had received  $P^{32}$  in the form of inorganic phosphorus. In this case the white pulp is blackened whereas the red pulp is only moderately affected (see Figs. 3 and 4). This agrees with the autoradiographs obtained by Gyllenstein & Ringertz (1954) after injection of inorganic  $P^{32}$ .

Autoradiographs of the liver and of the

mesenteric lymph node did not show any blackening after transfusion of living lymphocytes. The hepatic lymph node, on the other hand, was distinctly blackened, with the exception of the follicles, which thus showed less activity. (Since the photographic reproduction of these autoradiographs was not very successful no pictures are included here.) After an injection of inorganic  $P^{32}$ , on the other hand, the strongest blackening actually appeared just over the follicles. The mesenteric and hepatic lymph nodes were alike in this respect.

The autoradiographs of the bone marrow after living cells had been injected showed a weak, diffuse blackening. After an injection of inorganic  $P^{32}$  the autoradiographs of the bone marrow were intensely and evenly blackened.

Autoradiographs of the corresponding organs of the animal that had received the heated suspension did not show any activity at all.

### Interpretation of Autoradiographs

The main question is: In how far is the blackening observed due to radioactivity, still bound to the injected cells at the time of killing the animals? This question is best answered by taking each organ separately.

*Spleen.*—The control experiment with inorganic  $P^{32}$  shows that activity is chiefly localized to the white pulp. When living, labelled lymphocytes have been injected the blackening occurs mainly perifollicularly. The free  $P^{32}$  injected at the same time as the labelled cells is not enough to cause any appreciable blackening of the white pulp. This is also shown by the fact that the control experiment with cells destroyed by heating did not give any blackening. There can thus be no doubt that the blackening in the perifollicular region of the spleen is caused by  $P^{32}$  bound to transfused lymphocytes. That these cells are still intact is shown by an experiment by Weisberger, Heinle, Guyton & Storaasli (1951). They proved that  $P^{32}$  bound to destroyed lymphocytes was found to be distributed in the same way as inorganic  $P^{32}$  injected intravenously as soon as 10 minutes after the transfusion. If all the cells in our experiment had been destroyed after transfusion the result ought to have been the same as after an injection of destroyed cells, i.e. no blackening at all.

*Lymph nodes.*—The same argument applies to the liver lymph node as to the spleen. The fact that the blackening is differently localized after a transfusion of labelled cells than after an injection of inorganic  $P^{32}$  shows that the blackening is dependent on activity bound to the transfused cells.

*Bone marrow.*—Here the situation is somewhat different, there being a diffuse blackening both after a transfusion of living cells and after an injection of inorganic  $P^{32}$ . The fact that living cells gave a weak blackening and destroyed cells none at all does not justify any conclusions, since the amount of  $P^{32}$  injected had not been determined in this experiment.

The principal question, formulated in the introduction, whether the transfused thymus cells can be recovered in the perifollicular region of the spleen may thus be answered in the affirmative. Many research workers maintain that lymphocytes can be transformed into plasma cells (Roberts, Dixon & Weigle 1957; Kellsall & Crabb, 1958, and others) and this supposition is strengthened by the present investigation, which moreover strongly supports the hypothesis referred to above, concerning thymo-splenic integration in antibody formation.

### Comparison with Results Gained from Previous Transfusions of Labelled Thymus Cells

Concerning the spleen, this autoradiographic study confirms and complements results obtained in previous experiments on the transfusion of thymus lymphocytes labelled with  $P^{32}$  (Fichtelius, 1953 and 1957*a*). In these experiments, which were carried out on rats, the activity of the transfused cells could be observed also in the liver 24 hours after transfusion. It is therefore somewhat surprising that no blackening appears in the liver 48 hours after transfusion and that ordinary histological sections do not reveal any accumulation of lymphocytes. This is all the more

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remarkable as Osogoe & Hitachi affirmed that they had recovered transfused rabbit lymphocytes in the liver as late as 72 hours after transfusion to another rabbit. The recovery of labelled cells in the regional lymph node of the liver can pos-

sibly explain this fact. The transfused cells in the 48-hours experiment may already have passed the liver and accumulated in its regional gland. The significance of this find will be discussed in a different connection.

### Summary

Thymus lymphocytes labelled with radio-active phosphorus were injected intravenously from guinea-pig to guinea-pig. The recipient animals were killed after 48 hours and autoradiographs prepared from spleen, liver, hepatic and mesenteric lymph nodes and from bone marrow. The labelled cells were traced to the perifollicular regions of the spleen and non-follicular regions of the hepatic lymph node.

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## Survival of Cr<sup>51</sup>-labelled Red Cells from New-born Infants<sup>1</sup>

by SEVERINO FOCONI and STIG SJÖLIN

It has not yet been possible to define the mechanisms of the physiological anaemia of the new-born infant, and it will not be possible to do so until we are able to measure the rate of formation and the rate of destruction of the red cells during this period.

During the last decade some attempts have been made to estimate the life span of the red cells of new-born infants. In 1951, Mollison described the results of transfusing a mixture of placental blood and blood from adult donors to four anaemic premature infants. The survival of the red cells was determined by the method of differential agglutination (Ashby). Mollison concluded that "some, at least, of the red cells produced during late fetal life survive for a shorter time than adult red cells". In 1956 this conclusion was reinforced. No figures, however, could be given, but the measurements were said to suggest that the life span of fetal red cells is only slightly shorter than that of red cells of adults. Using the differential agglutination technique, after having transfused placental blood to four infants aged 11 days, and 6, 8, and 10 weeks, Seelemann (1954) came to the conclusion that fetal red cells had the same survival

time as red cells from adults. Vest (1959) used the same technique, and gave placental blood from four premature infants to four other premature infants. He interpreted the resulting curves as showing a life span of 70–90 days.

After the introduction of the Cr<sup>51</sup> method for estimation of red-cell survival it was natural to apply it to the problem of fetal red-cell survival. After having transfused labelled red cells from six full-term new-born infants to adult recipients, Hollingsworth (1955) found that the apparent half-life varied between 13 and 22 days, compared with a variation of 24 to 36 days in adults. Using the Cr<sup>51</sup> technique and following the survival after autotransfusion, Vest (1959) found an average apparent half-life of 24 days in 5 full-term new-born infants and 16 days in 7 premature ones. From very rough calculations Vest concluded that these figures give a mean cell life of 96 days in full-term new-born infants and 54 days in premature infants.

These investigators, using similar or totally different methods, have thus reached the similar conclusions regarding the life span of fetal red cells.

The investigation now described was

<sup>1</sup> This investigation was supported by grants from the Swedish Medical Research Council.

started in 1955 with the special aim of studying by the Cr<sup>51</sup>-technique the survival of red cells of premature infants, and of investigating the rate of chromium elution from intact red cells *in vitro*.

### Material and Methods

Red cells were obtained from the cord blood of 16 full-term new-born infants and from 11 premature ones. (Infants born more than 3 weeks before expected date have been regarded as premature.) The red cells were immediately labelled with Cr<sup>51</sup> according to the method of Gray & Sterling (1950) as modified by Brante (1956). The chromium content of the red cells of each individual is seen from the table. The labelled red cells were transferred to healthy, adult recipients, whose red cells were compatible with those of the infants with respect to the ABO blood-group system and to the D (Rh<sub>0</sub>) factor of the Rh system. In all cases the donor's red cells suspended in saline showed no agglutination when mixed with the recipient's serum. None of the recipients had previously received a blood transfusion. The radio-activity of a blood sample taken 20–30 minutes after the injection was regarded as 100 per cent. All Cr<sup>51</sup> half-times have been calculated from curves drawn on semilogarithmic paper.

In 11 of the 27 cases the survival curves initially showed a normal course, but after an interval varying from 5 to 15 days most of the labelled red cells suddenly vanished. Five of these cases have been described previously (Adner & Sjölin, 1957). Because similar abnormal survival curves have been found also when red cells from adult donors were used, and because they were interpreted as being due to immunization (Adner & Sjölin, 1957), these experiments have been excluded. Thus the final series consists of 10 full-term and 6 premature infants (Table).

In a series of 12 autotransfusions in 12 healthy male adults the mean apparent half-life was 27.5 days (S.D. = ± 2.25 days).

In order to be able to compare the Cr<sup>51</sup>-survival curves with Ashby survival curves

obtained from the literature, the Cr<sup>51</sup>-survival curves were corrected for a Cr<sup>51</sup> elution of the same magnitude as that found in red cells from adults. According to Hughes Jones & Mollison (1956), the half-time of the Cr<sup>51</sup> elution of red cells from adults is 64 days, i.e. about 1% of the remaining Cr<sup>51</sup> leaves the red cells per day. The individual experimental Cr<sup>51</sup>-survival figures were corrected for Cr<sup>51</sup> elution by means of the formula

$$y_c = y_m \cdot e^{k_{el} \cdot t} \quad (1)$$

where  $y_c$  = the corrected % survival at time  $t$ ,  $y_m$  = the measured % survival at time  $t$ ,  $k_{el}$  = the constant of elution of Cr<sup>51</sup> from the red cells = 0.01, and  $t$  = the time in days.

From the corrected Cr<sup>51</sup>-survival figures for the 12 healthy male subjects and the 10 full-term and the 6 premature infants, "smoothed" individual curves were drawn. From these, mean survival curves for the 3 groups were obtained. Mean differential agglutination curves for 6 full-term and 4 premature infants were calculated from individual survival curves taken from the literature. (Seelemann, 1954; Mollison, 1956; Vest, 1959). All mean curves are shown in Fig. 3.

In order to compare the chromium elution from red cells of new-born infants and adults, some experiments were performed *in vitro* under sterile conditions. Ten parts of whole blood were mixed with one part of acid citrate dextrose solution. After centrifuging this mixture, the plasma was saved and the red cells were labelled with Cr<sup>51</sup> as in the experiments *in vivo*. About 4 ml of the washed, packed red cells was then resuspended in 6 ml of their own plasma. The suspension was thoroughly mixed and divided into four equal portions in four test tubes. The content of one of these test tubes was immediately analysed for the Cr<sup>51</sup> activity of the red cells and the plasma, and for the degree of haemolysis (extinction of diluted supernatant at 5400 Å, Beckman spectrophotometer, model B). These values were used as blanks. The other three test tubes were placed in a refrigerator at +4°C for varying periods. The degree of haemolysis and the Cr<sup>51</sup> activity of the red cells and the plasma were then de-

terminated. The net  $\text{Cr}^{51}$  elution was calculated by subtracting the percentage of haemolysis from the percentage of  $\text{Cr}^{51}$  found in the plasma. Six experiments were performed with placental blood from six full-term new-born infants and six with blood from six adults.

### Results and Discussion

The apparent half-times ( $T_{1/2\text{app}}$ ) of the individual  $\text{Cr}^{51}$ -survival experiments are seen in the table. In Fig. 1 all the survival curves are presented. The  $\text{Cr}^{51}$ -method obviously gives shorter survival times for placental red cells than for red cells of adults when transfused to adult recipients. The mean apparent half-life of the red cells of the full-term new-born infants was 22.8 days. The red cells of the premature infants survived for a still shorter period, the mean apparent half-life being 15.8 days. These figures conform with those of Hollingsworth (1955) and Vest (1959).

TABLE 1.

Donor				Recipient	
Sex	Birth weight, g	Birth, weeks before or after expected date	Cr content, $\mu\text{g/ml}$ red cells	Sex	$\text{Cr}^{51}$ , $T_{1/2\text{app}}$ of fetal red cells
F	1540	-9	0.7	M	18
F	1850	-6	2.0	F	18
M	2170	-4	3.0	M	10
M	2430	-6	0.8	M	16
M	2580	-3	2.0	M	15
M	2680	-3	0.5	F	18
Mean 15.8					
M	2900	0	0.2	F	28
F	2900	-1	0.4	M	20
F	3200	+3	1.3	M	19
F	3270	0	1.0	M	25
F	3350	0	0.4	M	25
F	3360	+2	0.7	M	17
F	3440	0	0.4	M	24
M	3670	+2	2.0	M	25
F	4370	+2	1.0	M	24
M	4490	0	—	F	21
Mean 22.8					

The results of these investigations are not conclusive, however, until it is proved that the rate of elution of chromium is the same in fetal red cells as in red cells from adults. In fact data exist that are suggestive of a different rate of elution of chromium from placental red cells than from red cells of adults. In 1957 Suderman, White, & Israels showed that  $\text{Cr}^{51}$  was eluted more rapidly from cord haemoglobin solutions than from adult haemoglobin. Later these findings were confirmed by Erlandson, Schulman, Walden & Smith (1958). In dialysis experiments *in vitro*, however, these authors were unable to find any difference in the rate of elution of  $\text{Cr}^{51}$  from intact red cells of new-born infants and adults. Our own experiments, however, performed *in vitro* under different conditions, show that  $\text{Cr}^{51}$  is eluted more rapidly from fetal red cells than from red cells of adults (Fig. 2).

A comparison of our mean  $\text{Cr}^{51}$ -survival curves (having been corrected for a  $\text{Cr}^{51}$  elution of 1% per day) with the mean differential agglutination survival curves of new-born infants reported in the literature shows that shorter survival is obtained by the  $\text{Cr}^{51}$ -method (Fig. 3). The most probable explanation of this difference is that the fetal red cells lose  $\text{Cr}^{51}$  more rapidly than the red cells of adults also *in vivo*. If it is assumed that the differential agglutination curves give the true red-cell survival, it is possible to calculate the rate of  $\text{Cr}^{51}$  elution from fetal red cells *in vivo* by using formula (1), where  $y_c = \%$  survival of the mean Ashby curve at time  $t$ , and  $y_m = \%$  survival of the mean  $\text{Cr}^{51}$  curve at time  $t$ . A  $\text{Cr}^{51}$  elution of 2.2% per day is then found for placental red cells of premature infants. For placental red cells

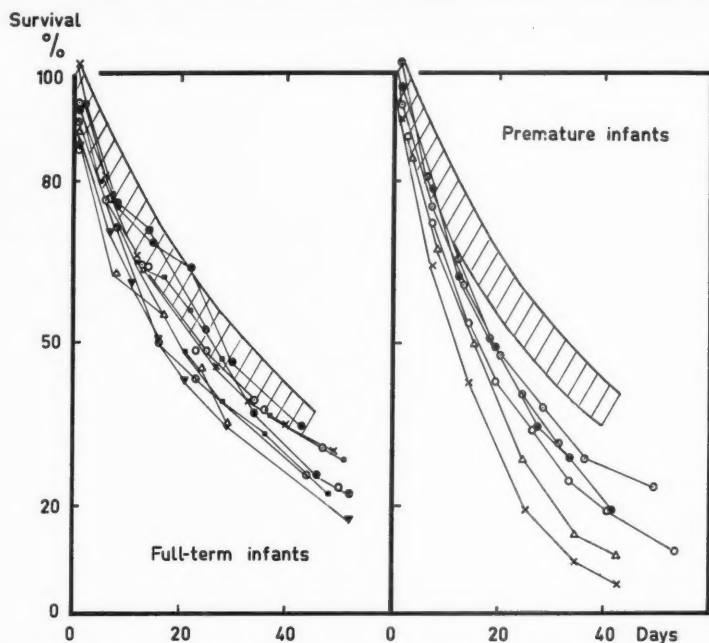


Fig. 1. Survival of  $\text{Cr}^{51}$ -labelled placental red cells from 10 full-term and 6 premature infants after transfusion to adult recipients. The hatched areas represent the normal variation in adults ( $\pm 2$  S.D.).

of full-term infants a  $\text{Cr}^{51}$  elution of 2.6% is found for the first 14 days, after which the rate of  $\text{Cr}^{51}$  elution is lower, at about 1.5%. Although these figures are approximate, being based on only a few Ashby curves with large variations both within and between the curves, they seem to indicate a more rapid loss of  $\text{Cr}^{51}$  from fetal red cells than from red cells of adults.

Because placental blood contains a high proportion of young red cells, the survival curves of placental red cells can be expected to show a falsely increased life span. Having this fact in mind and considering the results of the Ashby survival experiments it seems likely that fetal red cells have a shorter life span than red cells of adults. This statement is further

strongly supported by the pronounced difference found between the survival of red cells of full-term new-born infants and of premature ones, a difference which is shown both by the  $\text{Cr}^{51}$ -method and the Ashby technique (Fig. 3).

It therefore seems impossible to define exactly the life span of fetal red cells by using the  $\text{Cr}^{51}$  technique. Nor is the Ashby technique very practical for the purpose of determining the life span of fetal red cells. The method is laborious, has a low reproducibility, and does not permit autotransfusions.

In order to obtain exact figures for the life span of fetal red cells, further investigations using independent methods for determining the survival of fetal red cells

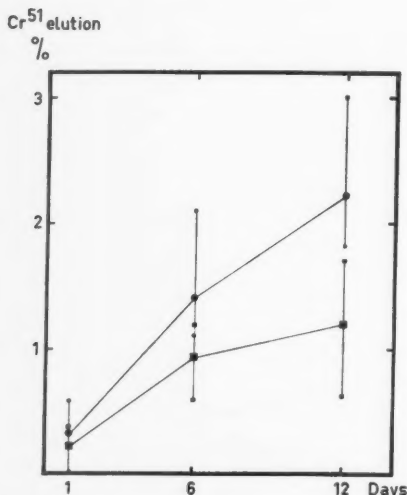


Fig. 2. Cr<sup>51</sup> elution in vitro from placental red cells of 6 full-term infants (●—●) and from red cells of 6 adults (■—■). The smaller dots indicate the extreme values.

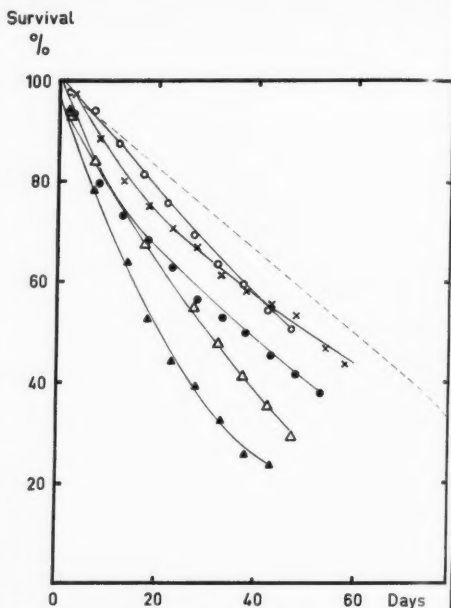


Fig. 3. Mean survival of Cr<sup>51</sup>-labelled red cells of 12 male adults after autotransfusion (x—x). Mean survival of Cr<sup>51</sup>-labelled placental red cells from 10 full-term (●—●) and 6 premature infants (▲—▲) after transfusion to adults. All the Cr<sup>51</sup>-survival curves have been corrected for a Cr<sup>51</sup> elution of 1% per day. Mean differential agglutination survival curves of red cells from newborn infants derived from the literature: 6 full-term infants (○—○) (Seelemann, 1954; Mollison, 1956) and 4 premature infants (△—△), (Vest, 1959). The dotted line indicates the survival of a homogeneous red-cell population with a life span of 120 days.

after autotransfusion and transfusion to other subjects seem necessary. Such investigations, using radio-active diisopro-

pylfluorophosphonate (DFP<sup>32</sup>) as a label of the red cells, are in progress.

### Summary

The survival of Cr<sup>51</sup>-labelled placental red cells of 10 full-term and 6 premature infants was studied after transfusion to adult recipients. The mean apparent half-life of the red cells from full-term infants was found to be 22.8 days, and that of red cells from premature infants 15.8 days (cf. 27.5 days for red cells from adults). Results obtained by others using the differential agglutination method have indicated a longer life span

than that corresponding to the Cr<sup>51</sup> half-times above mentioned. The most probable explanation of this difference seems to be that Cr<sup>51</sup> is eluted more rapidly from fetal red cells than from red cells of adults. This hypothesis is corroborated by the results of experiments in vitro on intact red cells.

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## Absorption of Labelled Iron in Infants Less than Three Months Old

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In recent years isotopically labelled iron has been used to estimate the amount of iron absorbed from the gastro-intestinal tract and also the effect of various imposed conditions and pathological states on this absorption. Recent reviews have been written by Josephs (1953 and 1958) and by Bothwell & Finch (1957). The following, well-established observations concerning the behaviour of the tracer in adults have emerged from these investigations: (1) The absorption of labelled food iron is of the order of 1-10%, which, on the assumption of steady-state conditions and a daily intake of iron of 10-100 mg roughly corresponds to the estimates of the daily excretion of iron of about 1 mg. This fact might be taken as a very rough indication that both estimates are correct and that the methods employed are adequate. (2) There is a quite considerable individual variation of iron absorption due to factors not yet known. It is improbable that this variation is due to the methods themselves. (3) The fraction of labelled

iron absorbed is inversely related to the amount of iron given and is also dependent on previous exposure of the gastro-intestinal tract to iron. These facts have been interpreted as implying that there is some sort of "mucosal block" operating. (4) The fraction of labelled iron absorbed is dependent on the form in which it is present in the test dose and also on the amount of ascorbic acid and cysteine taken at the same time.

Due to the three last-mentioned factors, a comparison of the absorption of tracer iron between different groups of individuals becomes difficult. In the work reported here we have studied the absorption of labelled iron in infants between 10 and 90 days of age on very similar diets. Approximately physiological amounts of iron containing the tracer have been given orally with and without food. The results will be discussed in relation to the results in adults referred to above and also with respect to previous investigations in infants.

<sup>1</sup> With the technical assistance of S. Foconi.

## Material and Methods

Twelve infants between 10 and 90 days old were investigated. They showed no signs of haematologic or digestive disturbances. Their nutritional status was good. The daily intake of iron is seen in Table 1 and was calculated from their food intake on the following principles: Breast milk was supposed to contain 1 mg/litre, cow's milk was supposed to contain 0.5 mg/litre and a special milk mixture (Findus Baby-välling, AB Findus, Sweden), sometimes used in the feeding, contained 30 mg/litre. The test dose consisted of 1 ml of a solution containing 10 or 20  $\mu\text{g}$  of Fe-56 as citrate and about 2  $\mu\text{C}$  of Fe-59.<sup>1</sup> At the same time, 0.05 g of carmine was given. The faeces were collected for the following 3 days or more. The stools and the diaper material were combusted in nitric acid and sulphuric acid and aliquots were taken for radioactivity measurements in a well-type scintillation detector with a counting efficiency of about 20 %. Aliquots of the test dose were measured at the same time. As a rule, all the activity that was recovered appeared in the carmine-coloured faeces, generally two days after the test dose was given. Fe-59 absorbed was taken to be Fe-59 given, minus Fe-59 recovered in the stools.

In the cases 1-3, the test solution was given between two meals and in cases 4-12, the test solution was administered with one of the ordinary meals.

## Results

The results may be seen in Table 1. The data show a definite, negative correlation between the amount of iron in the dose and the percentage of tracer iron absorbed. The decrease in tracer absorption with increasing load was 0.2%/μg and significantly ( $P < 0.01$ ) different from zero. However, even taking this source of variation into consideration, the variation between

TABLE 1.

Case No.	Age, days	μg of Fe given in test dose	Daily intake of Fe (μg)	% absorption of Fe-59 in test dose
1	16	20	500	71
2	59	21	30000 <sup>a</sup>	59
3	38	21	7900	96
4	81	50	300	38
5	10	60	360	56
6	34	70	420	66
7	51	110	660	23
8	22	110	550	91
9	88	120	600	15
10	33	165	825	56
11	63	250	750	36
12	90	450	2250	37

<sup>a</sup> This child received medicinal iron (Ferrosi pyrophosphis).

individuals is large, probably much larger than the error of the method itself.

If the transfer of iron across the mucosal barrier is a one-way process (see below), a value of the daily absorption of the iron can be obtained by multiplying the percentage of absorption of the tracer by the intake. This gives a mean value of 322  $\mu\text{g}$  per day (standard error 80  $\mu\text{g/day}$ ) for the cases 4-12. This value is probably slightly too low because the percentage of absorption was obtained from a test meal containing slightly more iron than the average meal, the relation between the percentage of absorption and the load being inverse.

## Discussion

Several points of interest arise from the data presented here. In the first place, the results should be compared with those obtained by Schulz & Smith (1957), who gave labelled iron with milk containing similar amounts of iron to infants between 4 and 52 months old. The tracer was ad-

<sup>1</sup> The absorbed dose of ionizing radiation from Fe-59 in these children was 200-400 millirads.

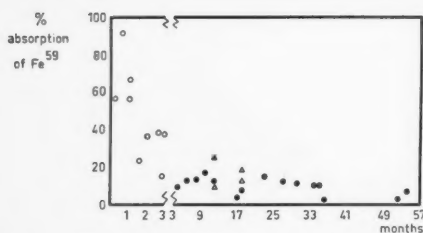


Fig. 1. The absorption of labelled iron as a function of age. Data from the present material (○). Data from normal (●) and iron-deficient (△) children (Schulz and Smith, 1957). Note change in scale of abscissa.

ministered either in the form of ferrous sulphate added to milk or in the form of *in vivo* incorporated milk iron. There was no difference in absorption between the two forms. The iron content of the test dose was 100  $\mu$ g. The mean amount absorbed was 10 % (range 2–17 %) and there was a pronounced tendency for the absorption to decrease with increasing age. *In vivo* labelled milk was also given to adult males, the test dose containing 350  $\mu$ g of iron and the mean percentage of absorption being 3 % (range 1–4 %). It is evident from a comparison of these data (see Fig. 1) with those obtained by the present study that the absorption of tracer iron from milk is considerably larger during early infancy than later in life.

Oettinger, Mills & Hahn (1954) found a much lower absorption (mean value 3.2 %, range 0.4–8.2 %) in newborn infants (2–4 days old), who received the labelled iron without food and with only 1  $\mu$ g of iron as ferrous chloride. However, in these studies, the amount of tracer absorbed was calculated from the amount given and the amount found in circulating red cells after 2–6 weeks. The assumption that all the absorbed iron is found in circulating red

cells at that time seems unwarranted and the figures for absorption must be considered too low.

The absorption of iron in the age group reported in this paper is considerably larger than the figures obtained from chemical balance studies on children of similar age and similar food and iron intake (approximately  $50 \pm 50$  micrograms per day for net retention of iron) (Wallgren, 1930; Snelling, 1933; Josephs, 1939; Sisson, 1951). This could be taken as evidence for excretion of iron in children of this group. However, another interpretation is also possible, namely that true absorption is not measured by the tracer experiments. The calculation of absorption of iron from the data on the absorption of the tracer in the conventional way, i.e. by multiplying the fraction of tracer absorbed by the dose, involves the unproven assumption that there is no isotopic exchange of iron across the mucosal barrier. This point seems to have been overlooked by investigators in this field.

The transmission of solute across a biological boundary must, in general, be looked upon as a result of two opposing currents of transport, the net flow being the difference between the two currents. A measurement of the flow of an isotope of the species under consideration in a specified direction can only give information of the flow in that direction, and the net flow remains undefined. Isotopic exchange denotes the simultaneous transfer, in opposite directions, of a labelled and an unlabelled particle. It must thus be borne in mind that isotopic transfer (in absorption experiments this is equivalent to disappearance of the tracer) can occur even in the complete absence of a net flow. Very few transfer mechanisms exist in which the flow is accomplished in only one direction, and only in such cases are measure-

ments of a tracer flowing in that direction sufficient for a complete description of the process.

If isotopic exchange occurs across the mucosal boundary, figures for absorption of iron as calculated in the conventional way will be too large. This also holds, as pointed out by Josephs (1953), for excretion of iron as measured by tracers.

It has generally been noted that the percentage of absorption of labelled iron decreases as the amount of iron in the test dose increases. This phenomenon, taken in the literature as evidence of "mucosal block", is evident also in the present age group. It has been argued that, because of the small iron excretion, the constancy of the body iron must be regulated through an absorption mechanism that in some way or other can "feel" how much iron the body needs. In the first place, however, as Josephs (1958) points out, the

constancy of the body iron has never been shown to exist. Secondly, the figures for the excretion of iron, while showing that it is small compared with the intake, by no means show that it is constant enough to necessitate the *ad hoc* hypothesis of a regulating power of the absorption mechanism. The behaviour of labelled iron in experiments where an iron load is given prior to the tracer dose and in experiments on iron depleted individuals (for literature, see Josephs, 1958) merely shows that the transfer of iron across the mucosal barrier is mediated by way of a reaction that shows saturation kinetics, i.e. that the iron competes for a limited number of reaction sites. This could be the result of a physical adsorption to cell walls or chemical binding to components in the lumen or in the cells. Such processes hardly deserve the term "mucosal block" or "regulating power".

### Summary

The absorption of radioactively labelled iron added to milk given to children less than 3 months old was investigated. The percentage of absorption of the tracer was found to be considerably larger than that observed at a later age. A comparison with figures for net retention of iron from milk in children of similar age indicates that excretion of iron takes place in these infants, or that true absorption of iron is not measured by the tracer method. Some difficulties in the interpretation of the conventional tracer absorption test are indicated. The phenomenon of "mucosal block" for iron is present also in the present age group. It is pointed out that simple physico-chemical phenomena might explain all data which have led to the concept of the "mucosal block" effect.

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## On the Use of Exchange Transfusion in Neonatal Thrombocytopenic Purpura

### Report of a Case

by ANDREAS KILLANDER

Although uncommon, thrombocytopenic purpura in the newborn is a well-recognized disease (cf. Morris, 1954; Stefani and Dameshek, 1955; Wintrobe, 1956). In most cases the mothers of the affected children have been known to suffer from either idiopathic or secondary thrombocytopenic purpura. Epstein, Lozner, Cobbey & Davidsson (1950) found that there was a high incidence of congenital thrombocytopenic purpura in infants borne by women with thrombocytopenic purpura. They suggested that the purpura in the newborn was caused by transplacental passage of a factor responsible for the thrombocytopenia in the mother. Such a factor was later found in the blood of some patients with idiopathic thrombocytopenic purpura (Evans, Takahashi, Duane, Payne & Liu, 1951; Harrington, Minnich, Hollingsworth & Moore, 1951; Tullis, 1956). Subsequently, platelet antibodies in the serum of both the mother and her infant were demonstrated in a few cases (Vandenbroucke & Verstraete, 1955; Selben, King & Duane, 1956). Thus, most cases of neonatal thrombocytopenia seem

to be due to transmission of antibodies from the mother to the infant. In this respect as well as in others to be discussed later, the disease resembles erythroblastosis foetalis.

The disease is self-limited, but the bleeding tendency, which may be pronounced during delivery and the first days of life, implies a great risk of profuse haemorrhages, sometimes resulting in fatal cerebral damage. Epstein *et al.* (1950) made an extensive review of all published cases of pregnancy complicated by purpura haemorrhagica and calculated the mortality at 26.1 % of the affected children. 8.7 % of the pregnancies ended fatally for the mother.

Different methods of treatment have been tried. Splenectomy of the mother prior to the delivery has reduced the bleeding tendency of the mothers but has not reduced the thrombocytopenia in the newborns (Epstein *et al.*, 1950). As regards the infant, blood transfusions seem to have been the standard treatment. Splenectomy has been performed in two cases but with disappointing results (cf. Morris, 1954).

Cortisone treatment had no effect on the thrombocytopenia in the infant described by Schoen *et al.* (1956).

In July, 1958, we treated a case of neonatal thrombocytopenic purpura. The general condition of the infant was good and there was no anaemia. However, pronounced thrombocytopenia and hypoprothrombinaemia were found and the mother was found to be thrombocytopenic (case report, see below). Considering different modes of treatment, we decided to use exchange transfusion for the following reasons.

The disease was possibly due to platelet antibodies transferred from the mother. Thrombocytopenia and bleedings which are sometimes found in severe cases of erythroblastosis foetalis usually subside following exchange transfusion. In the latter disease the exchange transfusion serves as a means not only of replacing sensitized Rh-positive cells by normal Rh-negative cells but also of removing circulating antibodies. By treating congenital thrombocytopenic purpura with exchange transfusion a similar result might be attained.

This paper contains a brief case report with special regard to the results of the exchange transfusion.

### Case Report

The patient was a boy, birthweight 3900 g, born the 22nd of July, 1958. The baby was the second child of a 26-year-old woman. She was found to suffer from tuberculosis of the right lung in September, 1953. Following treatment with streptomycine, para-aminosalicylic acid and iso-nicotinehydrazide, she improved and the disease was regarded as cured in November, 1954. In 1955 she was operated on because of acute appendicitis

and in 1956 she gave birth to her first child, a normal girl without bleedings. The delivery was normal. No abnormal bleedings had been noted during her different stays in hospital between 1953 and 1956. No platelet counts had been performed.

On the 21st of July, 1958, she was admitted to the Obstetrical Department three weeks after the calculated date of delivery. The next day she was given 0.2 g of quinine and 10 mg of K-vitamin orally. Twelve hours later she was delivered of an apparently healthy boy. The blood loss was 600 g.

On the 23rd of July, 11 hours after the birth, several small petechiae were found scattered over the whole body of the infant. A few echymoses were found on both arms. Further investigations revealed a platelet count of less than  $10,000/\text{mm}^3$  and a prothrombin-proconvertin value according to Owren of 15%. Hb was 23.2 g%, red cells 5.4 mill/ $\text{mm}^3$ , packed cell volume 70%, reticulocytes 10.2% and white cells 17,400 with a normal differential count. There were no neurological symptoms and no signs of intestinal bleeding. The spleen was not palpable. Both infant and mother had blood group A Rh+. A blood count of the mother revealed nothing abnormal except for an abnormally low number of platelets,  $74,000/\text{mm}^3$ .

The following day, the infant's platelets were still less than  $10,000/\text{mm}^3$  and an exchange transfusion was performed, using a plastic catheter and siliconated syringes. 530 ml fresh blood, collected in two plastic bags containing ethylene-diamine-tetraacetate were injected via the umbilical vein in 20 ml portions and the same amount was removed. The subsequent course is shown in the figure. The day after the exchange transfusion the number of platelets had increased to  $40,000/\text{mm}^3$ , but afterwards it began to decrease. No new bleedings were noted and the child was discharged on the 7th of August, 15 days after birth. Two weeks later the mother noticed some red, blood-like streaks in the infant's stool and he was readmitted to the hospital. On admission a few very small petechiae were noted on the

palate as well as on the body. No blood was detected in the faeces. The bone-marrow obtained by tibial puncture showed a reduced number of megakaryocytes with degenerative signs (B. Vahlquist). During observation for 9 days no bleedings, intestinal or cutaneous, were noted and the boy was discharged, 37 days after birth. During this second stay in hospital the platelet count remained low, less than  $15,000/\text{mm}^3$ . However, when seen in the Outpatient Department 49 days old, the platelet count had increased to  $60,000/\text{mm}^3$ . Since then the recovery has been uneventful.

In the meantime, the mother was thoroughly investigated in the Department of Internal Medicine. The platelet count, which had been studied seven times during four months, varied from  $52,000$  to  $87,000/\text{mm}^3$ . Otherwise the blood count was normal. The bleeding time was 2 minutes and the coagulation time 5.5 min. The sternal marrow was normal. The megakaryocytes were not reduced and appeared normal.

Before the exchange transfusion, blood was drawn from the infant and the serum kept frozen at  $-16^\circ\text{C}$  together with a serum sample from the mother. Four weeks later, the samples were sent to Dr. Kissmeyer, Aarhus, Denmark, for an assay of platelet antibodies. The samples were analyzed for complete antibodies according to Dausset and for incomplete antibodies with Coombs' consumption technique. In the mother's serum there was a very doubtful thrombocyte-agglutination in the Dausset test and a negative result in the consumption test. In the child's serum no antibodies were found.

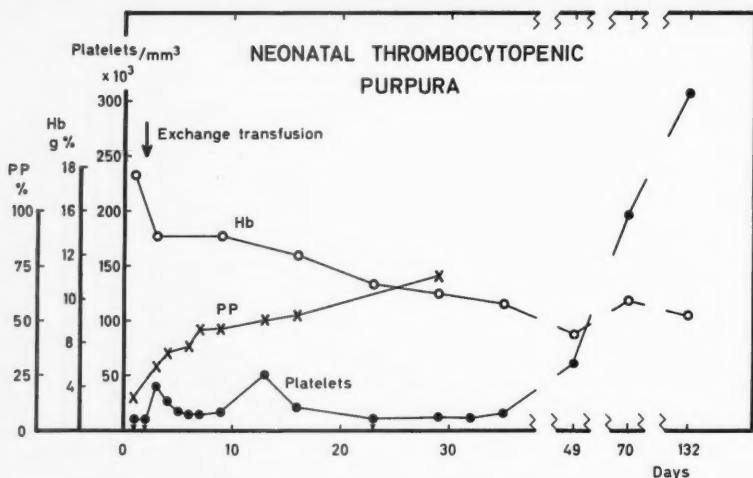
### Discussion

These two cases of thrombocytopenia in mother and infant show some unusual features. The mother had not suffered from abnormal bleedings before or during delivery and the disease would have remained undiagnosed if the infant had not shown a petechial rash after birth. The thrombocytopenia in the mother might

have been induced by the single dose of quinine before delivery, although this seems unlikely in view of the number of platelets being constantly below normal during the following four months. At present, it thus seems most likely that she has a mild form of idiopathic thrombocytopenia.

In the infant the thrombocytopenia disappeared rather slowly and a normal platelet count was not found until 70 days after birth. This might be due to the presence of antibodies not removed by the exchange transfusion. In the case described by Schoen *et al.* (1956), platelet agglutinins were demonstrated in the serum of the child six weeks after birth but not after twelve weeks. Following the exchange transfusion in this case, there was only a moderate and temporary increase of the platelet count. With the exception of one determination the platelet count then steadily decreased to the pretreatment level and at that occasion a few petechiae were noted as well as a questionable intestinal bleeding. The reason for this "relapse" and for the apparent changes of the megakaryocytes remains obscure. It may be noted that the eventual remission of the disease in the infant coincided in time with decreasing amounts of milk from the mother as she had practically no breast-milk left 70 days after the delivery.

The serological investigation failed to reveal the presence of platelet antibodies in the serum of the mother and the infant. This fact, however, does not disprove the possibility of an immunological reaction as the cause of the disease in the infant, in view of the well-known limitations of the methods of detecting platelet antibodies.



Following the exchange transfusion the bleeding tendency disappeared, but, of course, this effect cannot be ascribed to the exchange transfusion in this case. The same effect might have been obtained without any treatment at all. The hypoprothrombinaemia was corrected, which seems to be of value in this condition. The haemoglobin concentration decreased immediately after the exchange transfusion. There was no evidence of haemolytic disease due to blood group incompatibility. Probably, this effect is caused by the use of adult blood with a lower haematocrit than the infant's blood for the exchange transfusion.

Neonatal thrombocytopenic purpura demands special attention so that treatment can be instituted immediately after birth if necessary. Splenectomy seems to be contra-indicated in view of the fact that the disease is self-limited. Only small blood transfusions, providing a limited number of platelets, can be given to newborn infants. Of course, platelet transfusions may

be given, but theoretical objections may be raised against this treatment in those cases where the disease is due to the presence of platelet antibodies. Moreover, the preparation of platelet concentrates is a more complicated and time-consuming procedure than the drawing of blood for transfusion. There seems to be no reason for the use of corticosteroids in most of these cases. At present there is no evidence of antibody formation in the infant. Even if it were so, the effect of the treatment would probably be too late to prevent the bleedings in the severely affected infants. These facts, together with the arguments mentioned above, suggest the use of exchange transfusion in congenital thrombocytopenic purpura.

As the disease can be expected in infants born by women with idiopathic thrombocytopenic purpura it may be preferable to treat women with this disease according to the same principles as adopted for Rh-immunized women. Thus, it is suggested that they should be delivered in

hospitals with exchange transfusion units and preferably with laboratories for haematological serology. Apart from the therapeutic advantages, this would also en-

able more intense investigations of this interesting although rare disease to be undertaken.

### Summary

An exchange transfusion was performed in an infant with neonatal thrombocytopenic purpura. Both the infant and the mother presented some unusual features, which are briefly discussed. Although the disease is self-limited, the high mortality during the first days after birth indicates the use of effective therapy as soon as possible. Compared to other methods of treatment, exchange transfusion seem to be the best in several respects. It is suggested that women with idiopathic thrombocytopenic purpura should be delivered in hospitals with exchange transfusion units and laboratories for haematological serology.

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## Non-spherocytic Haemolytic Anaemia in Mother and New-born Infant

by LARS SÖDERHJELM

I have recently had the opportunity of observing a patient with non-spherocytic haemolytic anaemia which became worse during her two pregnancies, and megaloblasts were found in the bone marrow. At the time of her second delivery this woman's condition deteriorated greatly, and the child also showed haemolytic anaemia.

### Case Report

The patient is a woman born in 1920. She was well until 1940, when she was treated operatively for thyrotoxicosis. At operation the parathyroids were damaged, and she was obliged to take dihydrotachysterol during the following  $1\frac{1}{2}$  years. At the time of thyroidectomy she had no anaemia, but in 1941 she became acutely ill with pyrexia and anaemia, and was admitted to Skellefteå Hospital. On admission her haemoglobin was 43% and the erythrocyte count 2 million per cu. mm, with 8.9% reticulocytes. The osmotic resistance of the red cells was normal, the icteric index (Meulengracht) 1:14, and urobilin was present in the urine. There were no tape-worm eggs in the faeces, and no occult blood. The spleen was not palpable, the patient was not pregnant, and there was free hydrochloric acid in the gastric juice. There were typical megaloblasts in the bone marrow. On several occasions during her

stay in hospital the patient felt cold, and complained of nausea and abdominal pain, although the abdomen was soft and not tender. During these attacks the haemoglobin fell, and there was often some jaundice. Injections of liver preparations had no effect, and not until after repeated blood transfusion over a period of 3 months did the haemoglobin become stabilized at about 60% and the erythrocyte count at 2.8 million. The patient was then discharged.

In January, 1943, there was another brief episode of pyrexia and anaemia, the haemoglobin being 53%, the erythrocyte count 2.6 million, and the mean red-cell diameter  $8.2\ \mu$ . A fractionated test meal revealed spontaneous secretion with large amounts of acid. The bone marrow was normoblastic.

The patient remained well until February, 1950, when she was 8 months pregnant, when she developed anaemia. The haemoglobin was 38% and the red-cell count 1.35 million, with a reticulocyte count of 10-18%. The liver and spleen were not palpable, and the faeces contained no tape-worm eggs and no occult blood. The bone marrow was "highly cellular, and showed all the criteria of pernicious anaemia: diagnosis pernicious anaemia of pregnancy" (N. Nordenskjöld (Fig. 1). There was no obvious jaundice but on two occasions the patient had attacks of vague abdominal discomfort and nausea, at which times urobilin was present in the urine and the anaemia was more marked.

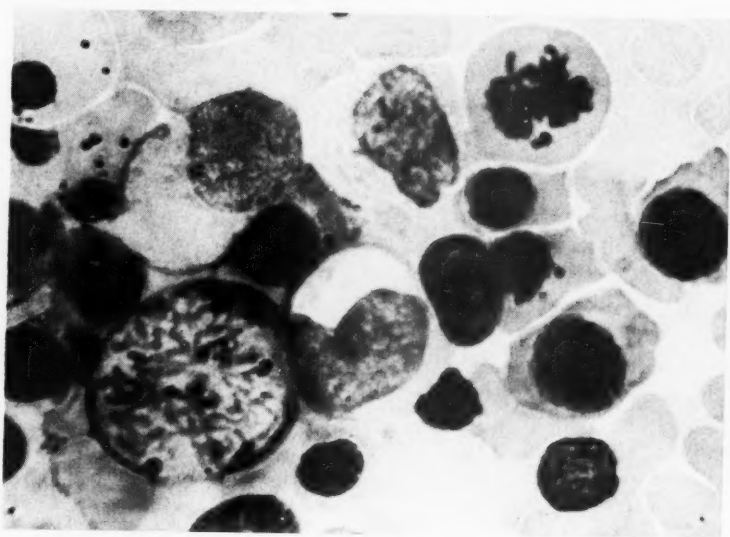


Fig. 1. Mother. Megaloblasts in bone marrow, 1950.

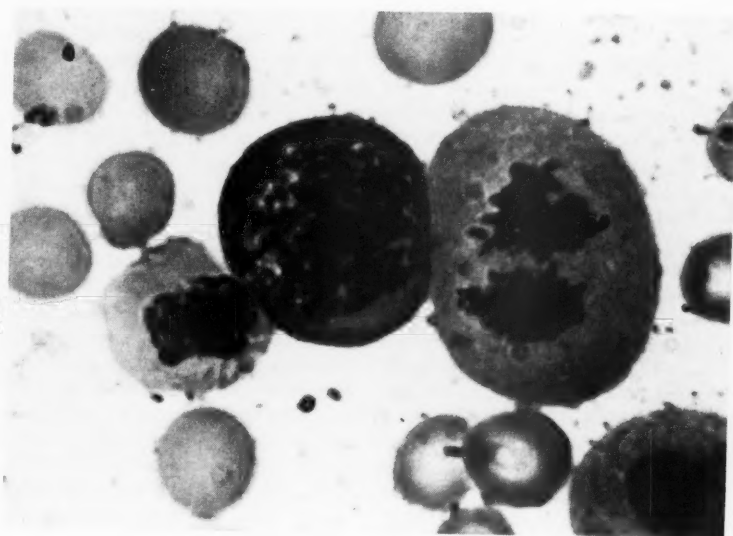


Fig. 2. Mother. Megaloblasts in bone marrow, Dec. 1955.

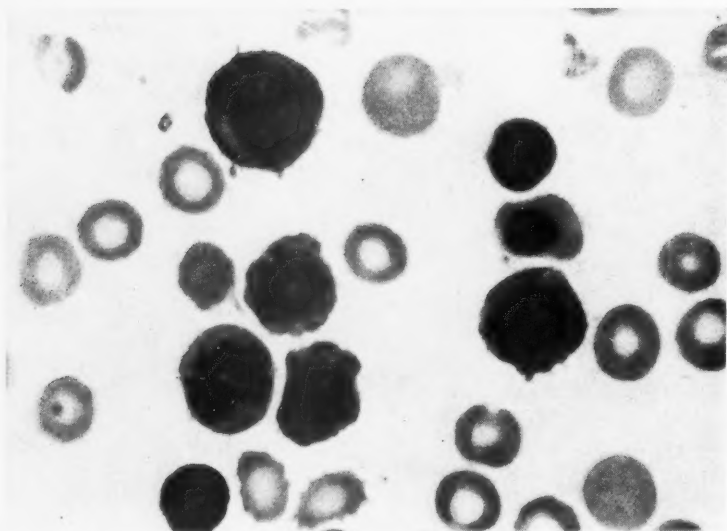


Fig. 3. Child. Megaloblasts in bone marrow, Jan. 1956.

Treatment with folic acid and liver preparations was without effect, but after several blood transfusions the patient was well enough to be discharged home. She had a normal delivery at home, and according to her the baby was healthy. She continued for a short time to take iron, liver, and folic acid preparations, but was soon completely restored, and on re-examination in January, 1955, the haemoglobin was 61% and the red-cell count 4.0 million.

The patient again became pregnant in March, 1955, and attended regularly the maternity welfare clinic. In October, 1955, the haemoglobin was 53%, and she was therefore given liver and iron preparations by mouth, and also folic acid. The haemoglobin rose temporarily, but in December, 1955, it was only 50%, and the erythrocyte count 2.4 million. The patient was therefore admitted to the obstetric ward, where she was given injections of liver preparations and intravenous iron, without effect. The osmotic resistance of the red cells was normal, and megaloblasts were present in the bone marrow (Fig. 2). On December 17th

she suddenly developed pyrexia and rigors, the haemoglobin fell to 36% and the erythrocyte count to 1.7 million, and there was deepening jaundice (Meulengracht index 1:80). She had to be given not less than 2 litres of blood before the haemoglobin began to rise again. On December 20th she gave birth to a boy.

At the time of the worst haemolytic crisis the reticulocytes constituted 45% of the red blood cells. The serum vitamin B<sub>12</sub> was 400  $\mu\text{g}/\text{ml}$  (Killander), and the serum iron only 80  $\mu\text{g}/100\text{ ml}$ , despite the extensive therapy. Tests of red-cell fragility gave the following data: haemolysis began at 0.58% NaCl, haemolysis was complete at 0.28% (but at the time these tests were carried out the patient had just received 2½ litres of blood within a period of three weeks, which may explain the diminished resistance). The Wasserman reaction was negative. The patient belonged to blood-group B, Rh (+) type Rh<sub>1</sub> Rh<sub>2</sub>, and "weak cold-agglutinin" was demonstrable in the serum; an indirect Coomb's test on the serum, using the child's blood cells as test cells was negative, how-

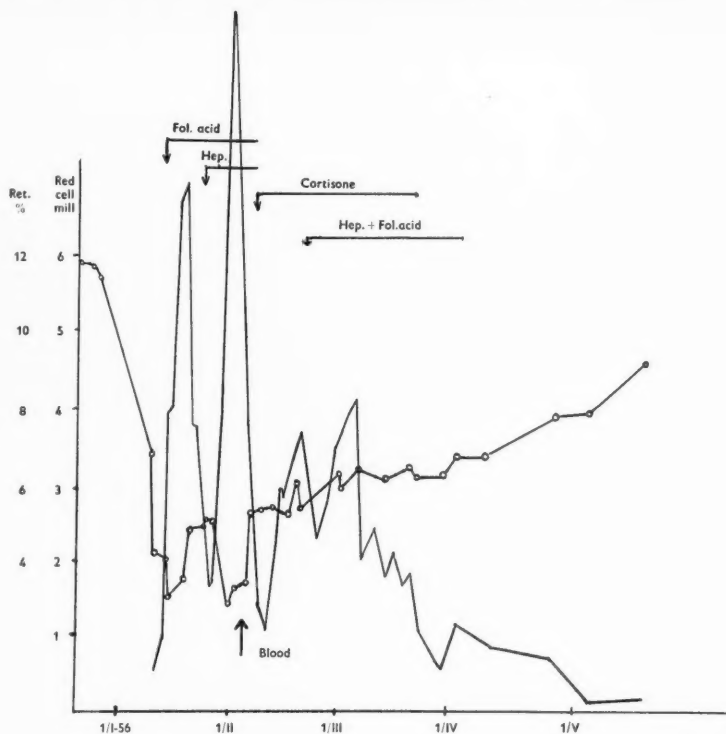


Fig. 4. Child. Reticulocytes (—) and erythrocytes (○—○) in relation to therapy. Hep. = liver preparation.

ever, and no other irregular antibodies were demonstrable" (B. Broman). The mother continued to receive treatment for a time with folic acid, and liver and iron preparations, and was soon restored. Repeated tests have shown the Hb to have remained at about 70%, and the erythrocyte count at about 3.5 million, the reticulocyte count at 0.3–0.9%, and the mean red-cell diameter 8.0  $\mu$ . She has had no haemolytic episodes since December, 1955.

The child was transferred to the paediatric ward for observation, and was bottle-fed on surplus breast milk from other mothers. He looked well, and his haemoglobin was 131%, and erythrocyte count 6.4 million. Jaundice soon developed, however, the spleen became

palpable, and the haemoglobin and red-cell count fell (Fig. 4). His blood group was 0 Rh (+), type Rh<sub>1</sub> Rh<sub>2</sub>, and "a direct Coomb's test was negative" (B. Broman).

A bone-marrow smear was richly cellular, and there was brisk but normal myelopoiesis. Erythropoiesis was fairly brisk and largely normoblastic, but not a few megaloblasts at different stages of development were distinctly recognizable (Fig. 3). The incidence of mitoses was greatly increased in the myeloid series, and also clearly increased in the erythrocyte series. Megakaryocytes were present in normal numbers (B. Vahlquist). The child's anaemia was interpreted as a megaloblastic anaemia, like the megaloblastic anaemia (?) of pregnancy of the mother, and

he was given vitamin C and folic acid. An increase in the reticulocyte count up to 27% and some improvement in the blood picture took place, but he soon deteriorated again (Fig. 4). New marrow puncture revealed "very brisk erythropoiesis, with mature cells predominating, but there was an increase in the number of basophil normoblasts. In addition there were still a few megaloblasts at different stages of maturity. The incidence of mitoses is markedly raised, and the chromosome pattern is strikingly blotchy" (Vahlquist). The red cells began to haemolyse at 0.48% NaCl, and haemolysis was complete at 0.30%. The patient was then given injections of a liver preparation, which produced a marked reticulocyte response (maximum 37%), despite the fact that blood tests before liver therapy showed 450  $\mu$ g of vitamin B<sub>12</sub>/ml. (Killander). The haemoglobin and red-cell count fell quite soon again, however, and a haemolytic component was suspected. The child received a transfusion of 80 ml of blood, and at the same time cortisone, which resulted in a new positive reticulocyte response (maximum 16%) and increasing haemoglobin and red-cell count. Repeated tests over a period of more than 2 years have subsequently revealed normal values, with about 0.6% reticulocytes and mean red-cell diameter of 8.0  $\mu$ , but the serum iron has been a little low (c. 38  $\mu$ g/100 ml).

### Discussion

Acute haemolytic anaemia is not uncommon among young subjects, especially during pregnancy (3, 4, 6, 14). Cases have been described quite long ago, but the condition has become more widely known largely through the work of Lederer.

The onset is acute with pyrexia, nausea and vomiting, headache, and sometimes also abdominal pain and diarrhoea, and at the same time anaemia develops and may progress very rapidly. As a rule there is leukocytosis and marked reticulocytosis

with nucleated red cells in the circulating blood, and in the bone marrow there is active erythropoiesis. The red-cell fragility is usually normal, but may be increased (10). The patient may recover after a single blood transfusion, but recidivating cases requiring many transfusions are common. The condition is thought to be due to haemolysins or agglutinins, which are not always demonstrable, however, and the treatment may include blood transfusion, ACTH and cortisone, and possibly even splenectomy (5, 15).

Attacks of acute haemolytic anaemia are sometimes seen during pregnancy, and never appear at other times. In such cases the anaemia usually disappears after delivery (1, 2, 11, 17). Some reports have appeared of spherocytic haemolytic anaemia that has become worse during pregnancy (8, 12), and a few cases of megaloblastic anaemia combined with haemolytic anaemia during pregnancy are published (7, 9, 13).

In isolated cases of pernicious anaemia of pregnancy the child has also shown megaloblastic anaemia (18, 19), whereas in those cases of acute haemolytic anaemia in which the pregnancy has been successful the infants have been unaffected.

In our case, the mother had recidivating haemolytic anaemia with normal red-cell fragility and no signs of splenomegaly. Her anaemia fulfilled all the requirements of non-spherocytic haemolytic anaemia, even though Coomb's test was repeatedly negative and no agglutinins other than a weak cold-agglutinin were demonstrable in the serum. Cold-agglutinins are so commonly found among healthy subjects, however, that no significance can be attached to their presence (16). During the

patient's pregnancies the anaemia was aggravated, and despite intensive treatment with iron, folic acid, liver preparations, and vitamin B<sub>12</sub>, nothing less than repeated blood transfusion would improve her condition. Her first child was born at home, and is said to have been normal, whereas the second had clear-cut anaemia

with a reticulocyte response to treatment with folic acid, with liver preparations, and with cortisone, and improvement in the haemoglobin and erythrocyte count. It is therefore possible that the mother had both haemolytic anaemia and pernicious anaemia of pregnancy producing a similar state in the child.

### Summary

A case is described of non-spherocytic haemolytic anaemia in a woman. The condition was aggravated during her two pregnancies, and exhibited some megaloblastic features. The first child was healthy, but the second developed anaemia with reticulocyte response first to folic acid, then to a liver preparation, and finally to cortisone and blood transfusion. Haemolytic anaemia was undoubtedly present, but the child also had folic-acid and vitamin B<sub>12</sub> deficiency.

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## Fatal Bacterial Infections in the Antibiotic Era

by RUTGER LAGERCRANTZ

The mortality in bacterial infections among Swedish children has markedly decreased. Between 1911 and 1950 this mortality dropped from 9.1 to 0.7 per thousand among infants and from 2.3 to 0.08 per thousand in children between one and fourteen (6). This decrease is probably mostly due to the better living conditions and care. Sulpha-drugs and antibiotics have made it possible to treat most bacterial infections. In spite of these improvements children still die from such infections.

In this paper some fatal infectious cases from a children's hospital will be analysed. This analysis of the diagnosis and treatment of infectious disease may be interesting as an illustration of the changing problems in pediatrics.

### Material

The Pediatric Clinic of the Karolinska sjukhuset, Stockholm, has furnished the material. In this Department 13,984 patients were admitted from July 1, 1951, to December 31, 1957. Four hundred and twenty-four patients died. In 63 patients the main cause of death was registered as bacterial infections. Twenty-nine of these patients had a severe congenital malformation or malignant disease. The infectious disease was, in these cases and in ten cases of cystic fibrosis of the pancreas,

of secondary importance for the fatal outcome. An example of a patient with a congenital abnormality in whom the infection was of decisive importance follows:

Girl born April 24, 1955. Fourth child to healthy parents. Pregnancy, delivery and birth weight normal. Patient cyanotic since birth. X-ray revealed ileus due to atresia of lower ileum, verified at operation. After ileostomy, oxytetracycline was administered intravenously 25 mg four times daily. Four days later fever and multiple subcutaneous abscesses developed. A few days later the patient became lethargic, hepatomegaly occurred and the patient died. Ante-mortem cultures from an abscess and post-mortem cultures from blood and several organs produced *Staphylococcus aureus* which were resistant to penicillin and oxytetracycline.

In twenty-four cases a bacterial infection was considered the primary cause of death. As seen in Table 1, most patients were infants, nine were below one month of age.

In eighteen cases the patients were severely ill (some moribund) when the infection was diagnosed and treatment started. In three cases of capillary bronchitis and in one case of meningococcal meningitis the duration of the disease was stated to have been only a few hours. Four of the patients with respiratory infection in the neonatal period probably had their infection at least one day before treatment. The first signs were lethargy and/or respiratory distress. In five cases of meningitis treatment was started late as lumbar puncture was performed twelve hours or more after onset of disease.

TABLE 1. *Diagnosis, age and time of diagnosis in 24 fatal cases of primary bacterial infections in the Pediatric Clinic of Karolinska sjukhuset.*

Number within brackets = total number of cases registered.

Diagnosis	Age-group			Late diagnosis	Total
	< 1 mo.	1-12 mo.	1-14 yrs.		
Bronchopneumonia } (1608)	5	2	1	6	8
Capillary bronchitis }		4		4	4
Sepsis (6)	1	1		2	2
Meningitis (66)	1	3	5	5	9
Tetanus (1)			1	1	1
Total	7	10	7	18	24

The two cases of septicemia were caused by *Staphylococcus aureus*. One of these cases had the following course:

Girl born February 2, 1955. Pregnancy and delivery normal. Birth weight 2650 g. On eighth day of life (while still in the maternity hospital) stuffy nose and fever. Nose and throat culture revealed colon bacilli and alpha-hemolytic streptococci. X-ray of the lungs showed atelectasis (bronchopneumonia?). Tetracycline 25 mg four times daily was administered. Three days later better but next day lethargic. On twelfth day of life general condition poor, skin hemorrhages, liver enlargement, blood culture revealed growth of *Staphylococcus aureus* resistant to penicillin and tetracycline but sensitive to chloramphenicol. Tetracycline was replaced by chloramphenicol parenterally. The patient expired the following day. Autopsy revealed sepsis; *Staphylococcus aureus*, apparently of the same type as isolated intra vitam, was cultured from the blood and from several organs post mortem.

The patient who died from tetanus was a ten-year-old girl not vaccinated against the disease.

Every year between seventy and ninety premature are admitted to the Clinic. In only two of these patients was the primary cause of death considered to be of bacterial origin (bronchopneumonia).

Eleven out of the twenty-four cases in Table 1 the children apparently acquired their infection in the Clinic (in the nursery or the

wards). In altogether fourteen cases *Staphylococcus aureus* was isolated from nose and/or throat. Staphylococci were apparently the etiologic agent in the two cases of septicemia in Table 1 and in the operated case referred to above.

### Discussion

The patient material is not quite representative of Stockholm. The Clinic admits a relatively large number of patients with special, non-infectious diseases such as allergic disorders and congenital malformations of the heart. Many patients with severe infectious diseases in Stockholm are sent to the Hospital for Infectious Diseases.

The number of fatal cases registered as caused by bacterial infections is probably lower than it should be because the diagnosis of infections can be difficult especially in the neonatal period. An infection at this age can run without classical signs as earlier stressed by Vahlquist (8). This is demonstrated by some of our patients, who were afebrile in the early course of the disease. A common cause of death in newborns is respiratory insufficiency combined with atelectasis with or without hyaline membranes. These patients usually

soon become infected. The infection often plays a decisive role in the fatal outcome. These cases are not included in this material.

Our patients have not been investigated for viral infections. In Sweden, Kjellén, Sterner & Svedmyr have demonstrated the combined viral and bacterial origin of some respiratory infections of childhood (3).

Even if the material is not quite representative of Stockholm, it reflects the declining mortality in bacterial infections in Swedish children (6, 8). Death due to tuberculosis and primary gastro-enteritis, which was not observed in this material, is extremely rare in all Swedish children's hospitals (6). The declining frequency of severe bacterial infections is easily observed even in general practice in Sweden.

Most fatalities were observed in infants. There are many reasons why infections run a severe course in infancy (8, 9). In our material most infants acquired their infection in the Clinic. In many parts of the world an increasing number of hospital-acquired infections in newborns and their families has been reported (2, 4, 5). They are often caused by special strains of colon bacilli or antibiotic-resistant *Staphylococcus aureus*. Our Clinic has not had any severe epidemics in the nurseries, but the above case histories illustrate that hospital infection with resistant staphylococci is also a problem with us.

The low mortality in infections among the prematures is remarkable. Some prematures died early in respiratory distress and were probably infected. As already mentioned, these cases are not included in this material. After the second week of life most deaths in prematures are considered to be due to infections (1). In this

age period only two prematures were registered as having died from bacterial infection (bronchopneumonia). The low incidence of infections in this group has probably many causes. We have had no severe epidemics of staphylococcal or colon bacilli infections. We have a well-planned and not overcrowded ward and specially trained and supervised personnel. In the ward for the prematures and non-infected infants no prophylactic treatment with antibiotics is given. We agree with the bacteriologists and clinicians who stress that nosocomial infections cannot be prevented in this manner and only become more difficult to treat. Even in surgery we seldom recommend prophylaxis with antibiotics. The first case history above illustrates the failure of such treatment.

We believe that *treatment* with antibiotics should be given for broader indications in the neonatal period than in older children as early diagnosis of infection might be difficult in this age-group and the infections are more apt to be severe or fatal. Thus all the newborns with respiratory distress are treated with broad-spectrum antibiotics, which otherwise are sparsely used in the department (2). It is very important that treatment when necessary is rapidly changed according to results of serial bacteriological investigations. This unfortunately was not done in the patients whose case histories were presented above. We now have valuable drugs even against staphylococcal infections resistant to the common antibiotics (7).

As already mentioned, this material does not give the complete picture of the fatal infections in our Clinic. To obtain more reliable data, bacterial examinations

should be performed during post-mortem examinations in all cases which have had definite or suspicious signs of infections. When the results of these investigations are obtained it is possible to make a complete epicritical evaluation as in the two cases reported here. It is probably of great importance that such cases are presented and discussed at a conference for the hospital staff and personnel (2, 4, 5, 7). This might be one way to lower the incidence of hospital-acquired infections.

### Summary and Conclusions

Some fatal cases of bacterial infections from a children's hospital are presented and analysed. The total mortality in such diseases is now low in Sweden. In order to

reduce it still further, the following precautions are suggested:

1. Early clinical and bacteriological diagnosis (especially in infants regarding capillary bronchitis and meningitis).
2. Bacteriological examinations (sensitivity tests included), repeated in cases with poor response to treatment. Early report of results.
3. Good hospital hygiene. The hospital should not be dangerous for the patients.
4. No prophylaxis with antibiotics. Limited use of broad spectrum antibiotics for treatment.
5. In fatal cases with proven or suspected infections bacteriological examinations post mortem are important for complete evaluation.

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## Evaluation of the Recovery of *Staph. aur. pyogenes* from the Faeces of Children.

by G. LAURELL, L. PHILIPSON and Å. GYLLENSWÄRD

A number of investigators have reported the association of *Staph. aur. pyogenes*<sup>1</sup> with infantile diarrhoea; but the evidence for its pathogenicity in the gastro-intestinal tract is still debated (4, 5, 6, 10, 11, 12, 13). These organisms have also commonly been recovered from the stools of asymptomatic infants (6, 12), and the pathogenicity of these staphylococci in the gastro-intestinal tract has therefore been linked with their enterotoxin-producing capacity (14).

Changes in the gastro-intestinal flora after treatment with antibiotics, especially of the tetracycline type, constitute an important complication, since they may result in disease (2, 8). Both *Staph. aur.* enteritis and pseudomembranous enterocolitis may probably be ascribed to this mechanism, but the latter seems to be relatively uncommon in children (1). Thus antibiotic therapy may result in overgrowth of a strain of staphylococci that has developed resistance during therapy, or in hospital infection with a naturally resistant strain differing from the original strain in phage-pattern and sensitivity towards antibiotics.

From the clinical point of view it seems important to elucidate whether the presence of staphylococci in the faeces of children is an indication for antibiotic treatment, irrespective of the presence of diarrhoea and whether the appearance of a resistant strain during or after treatment should occasion a change in the antibiotic administered.

The study presented was designed to investigate these problems.

### Clinical Material

The investigation was carried out from March 1954, to December, 1956. Only children from whom *Staph. aur.* had been isolated from faeces on some occasion were included. Since not all children were examined on admission, the series represents a random sample. Altogether 95 children were included. Of these, 14 were admitted to hospital with the diagnosis of infantile gastro-enteritis, and a further 18 cases developed hospital infection during their stay. The remaining 63 children had no symptoms from the intestinal tract.

*Antibiotic treatment.*—Most of the children with enteritis either on admission or acquired in hospital were treated with oxy-tetracycline (Terramycin) in doses of 25

<sup>1</sup> *Staph. aur. pyogenes* has been used for coagulase-positive strains of *Staph. aur.*, and they are hereafter termed staphylococci.

mg/kg body weight divided into 4 daily doses. Tetracycline (Achromycin) was used in some cases, in the same dosage. Some of the children with diarrhoea received no antibiotic treatment. The 63 children with no diarrhoea were also treated with antibiotics of tetracycline type when antibiotics were indicated for other reason, for comparison with the "enteritis" groups, but not all of these cases received antibiotics.

### Methods

**Faeces.**—Samples of stools were ordered to be taken on admission to hospital, and before and after antibiotic treatment. Unfortunately, however, specimens were not obtained to the extent desired. Cultures were started within 12 hours of sampling.

**Bacteriological technique.** The following media were used. (1) Endoagar plates, (2) ordinary agar plates containing 10% of defibrinated sheep's blood, (3) phenol-mannite agar plates (Chapman, 3), (4) Kauffmann broth for enrichment of *Salmonella* species, and (5) desoxycholate agar plates, for secondary culture from the Kauffmann broth.

The diagnostic equipment thus covered salmonella, pathogenic coli, and staphylococci.

Staphylococci were isolated from the sheep's blood agar and the Chapman plates. They were read at 24 and 48 hours after incubation at 37°C. The amount of staphylococci recovered was graded from + to +++ (+ meaning <10 colonies; ++, 10–50 colonies; and +++, >50 colonies). All strains with a yellowish pigment were tested for

coagulase by the tube method, and only coagulase-positive strains were classed as *Staph. aur. pyogenes*.

**Phage patterns.**—Phage-typing of the staphylococci was carried out to some extent, and was kindly performed by Dr. G. Wallmark, State Bacteriological Laboratory, Stockholm, Sweden. The phage strains used and the technique has been described previously (15).

**Sensitivity tests.**—All strains of *Staph. aur.* were tested for resistance to the commonly used antibiotics (sulphonamides, penicillin, erythromycin, streptomycin, chlortetracycline, oxytetracycline, and chloramphenicol). The sensitivity tests were carried out according to the disk method described by Ericsson *et al.* (7). Resistance to antibiotics was assessed by the inhibitory concentration in vitro, the following concentrations indicating resistance. Sulphonamides, 6 mg%; penicillin, 2 IU/ml; erythromycin, 4 µg/ml; streptomycin, 12 µg/ml; chlortetracycline, 4 µg/ml; oxytetracycline, 4 µg/ml; Chloramphenicol, 6 µg/ml. Strains inhibited by these concentrations are termed sensitive in this investigation, and those showing growth are classed as resistant.

### Results

#### *Characteristics of the Staph. aur. pyogenes strains isolated*

The sensitivities to antibiotics of the tetracycline type are shown in Table 1. Only 19.7% of the strains isolated on admission were resistant towards antibiotics

TABLE 1. *Sensitivity to antibiotics of the tetracycline type of Staph. aur. pyogenes isolated from the faeces of children on admission and after one week or more in hospital.*

No. of strains on admission			No. of strains after one week or more in hospital			Strains resistant on admission, per cent	Strains resistant after 1 week or more in hospital, per cent
Sensitive	Resistant	Total	Sensitive	Resistant	Total		
49	12	61	12	48	60	19.7	80.0

TABLE 2. *Phage patterns of Staph. aur. pyogenes strains isolated from faeces of children on admission and after one week or more in hospital.*

Phage patterns	No. of strains on admission	No. of strains after one week or more in hospital
KS 6	1	—
6/47/819/1034	2	2
6/47/1034	1	—
6/47	1	—
47/819/1034	1	3
47/1034	—	1
819	—	3
155	—	1
166	2	—
Not typable	11	13
Total number of strains phagetyped	19	23
Per cent of strains not typable	57.8	57.0

TABLE 3. *Staph. aur. pyogenes and their resistance to antibiotics of the tetracycline type in the faeces of children WITH DIARRHOEA on admission or acquired in hospital.*

	No. of children with <i>Staph. aur.</i> in their faeces	No. of strains before treatment Samples taken at		No. of strains after treatment	No. of children in whom staphylococci were eliminated by treatment	No. of children not treated
		1-14 days in hosp.	> 14 days in hosp.			
Hospital infections	18	7/3 <sup>a</sup>	1/7	1/10	2	3
Diarrhoea on admission	14	9/1	—	1/4	5	2

<sup>a</sup> Numerator denotes strains sensitive and denominator strains resistant to antibiotics of the tetracycline type.

of the tetracycline type, but 80 % of the strains isolated after one week or more in hospital showed resistance. Two strains of pathogenic coli were isolated in this material together with *Staph. aur.*, both type 0111, B4. No symptoms were observed in the children with these strains. No *Salmonella* organisms were recovered from the children examined.

The phage patterns of 42 strains tested are shown in Table 2. The strains isolated from the epidemic described later are not included in the table. Many of the strains could not be phage-typed; and of the strains that reacted with phages no clear difference in pattern was observed between strains isolated at the time of admission and those recovered after one

TABLE 4. *Staph. aur. pyogenes and their sensitivity to antibiotics of the tetracycline type in the faeces of children WITHOUT DIARRHOEA.*

Total no. of children	No. of child- ren not treated	No. of strains before treatment		First course of treatment			Second course of treatment		
		Samples taken at 1-6 days in hosp.	> 6 days in hosp.	No. of strains after	No. of children in whom staphy- lococci were elim- inated	No. of cases showing change in phage- type	No. of strains after	No. of children in whom staphy- lococci were elim- inated	No. of cases showing change in phage- type
63	13	32/8 <sup>a</sup>	3/5	4/26	6	4	0/7	1	2

<sup>a</sup> Numerator denotes strains sensitive and denominator strains resistant to antibiotics of the tetracycline type.

week or more in hospital. All strains except one that reacted with phages belonged to phage group III according to Williams *et al.* (16).

*Change in antibiotic sensitivity of staphylococci from children with diarrhoea and children with no such symptoms*

Among the children with diarrhoea either on admission or due to hospital infection, a marked increase in resistant strains was observed after tetracycline treatment (see Table 3). Effective treatment in the bacteriological sense, viz. elimination of staphylococci from the faeces, was achieved in 5 children who had had diarrhoea on admission but only in 2 children with hospital infection. Clinically, most of the children with diarrhoea improved on treatment, in spite of a high incidence of resistant staphylococci in the faeces after treatment.

The staphylococci recovered from the children with no diarrhoea showed similar characteristics (see Table 4). Initial treatment with antibiotics of the tetracycline type upon various indications resulted in marked increase in resistant strains, and

elimination of the staphylococci was achieved in only 6 children. In 4 of this group of children the change in resistance was accompanied at the same time by a change in phage-type. After a second course of treatment with antibiotics of the tetracycline type no sensitive strains could be isolated, and in two cases *Staph. aur.* was eliminated. Change in phage-pattern of the staphylococci between the first and second courses of treatment was observed in 2 cases.

*Change in type of staphylococci induced by treatment or occurring spontaneously*

The previous results suggested that the reappearance of staphylococci in the faeces may have been due to a substitution of the strain due to treatment, but this may also occur spontaneously without antibiotic treatment. Table 5 shows the number of strains in which the phage pattern and/or antibiotic sensitivity differed from those of the original strains in children treated and untreated and with and without diarrhoea. As expected, there was a marked increase in resistant strains after antibiotic treatment, but in some children the strains

TABLE 5. *The substitution or elimination of strain of Staph. aur. in the faeces of children induced by antibiotics of the tetracycline type or occurring spontaneously.*

No. of strains of <i>Staph. aur.</i> differing from the original strain		No. of children in whom <i>Staph. aur.</i> disappeared from faeces	
After treatment	Spontaneously	After treatment	Spontaneously
<i>With diarrhoea—32 children examined</i>			
0/10 <sup>a</sup>	1/2	5	2
<i>Without diarrhoea—63 children examined</i>			
0/14	4/1	5	2

<sup>a</sup> Numerator denotes strains sensitive and denominator strains resistant to antibiotics of the tetracycline type.

were replaced by others even without treatment. These new strains from untreated children seem to be less resistant than those appearing after antibiotic therapy.

*A minor epidemic of diarrhoea probably caused by staphylococci*

This outbreak occurred in the infants' ward. One child developed diarrhoea on November 26th and the second case was observed on December 2nd. Within 2 weeks further 6 children were affected, and in all 8 children were involved. Clinically the enteritis was mild, 5–7 diarrhoeal stools being passed per day. As seen in Fig. 1, strains of varying sensitivity to antibiotics were isolated at the onset of symptoms in some of the children, but all but one of these strains reacted with phage 166. Thus in spite of different sensitivity pattern these strain may be identical. It is of interest that all the strains reacting with phage 166 did so only in high concentrations of phage and the non-typable strain isolated may therefore be identical to the others. After treatment,

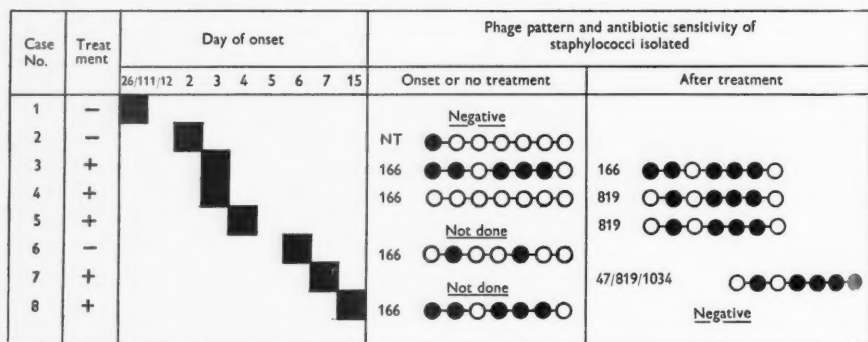


Fig. 1. An epidemic of enteritis probably caused by staphylococci within a paediatric ward unit. Antibiotic sensitivity pattern is indicated by seven linked circles representing, in this order,

- sulphonamide
- penicillin
- erythromycin
- streptomycin
- chlortetracycline
- oxytetracycline
- chloramphenicol
- , resistant; ○, sensitive. NT = Not typable.

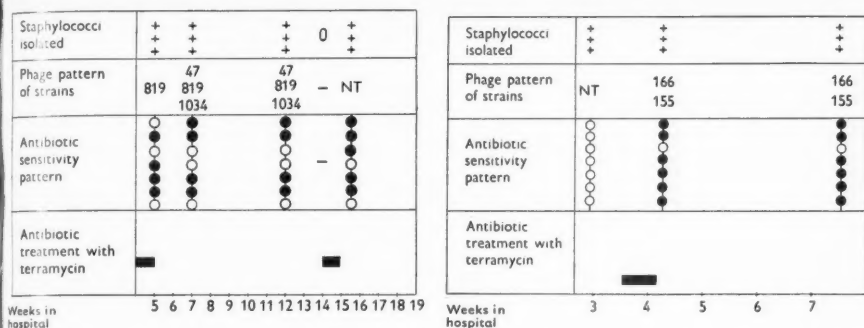


Fig. 2. Two cases illustrating the substitution of strains of *Staph. aur.* after treatment or occurring spontaneously. NT = Not typable. Antibiotic sensitivity pattern is indicated by seven linked circles representing, in this order,

- sulphonamide
- penicillin
- erythromycin
- streptomycin
- chlortetracycline
- oxytetracycline
- chloramphenicol
- , resistant; ○, sensitive.

however, resistant strains were isolated from most of the treated children, and in some cases the phage-patterns differed from those of the original strain. Treatment was clinically effective in all cases, and no diarrhoea was present after 4 days of treatment.

#### A selection of cases to illustrate the results

Further to illustrate the fact that treatment with antibiotics of the tetracycline type or even no antibiotic treatment may result in substitution of strains of staphylococci in the faeces, two cases will be described.

The first case (Fig. 2) is that of a 1-week-old girl admitted to hospital for a cardiac examination owing to situs inversus of all internal organs and defects in the atrial septum and great pulmonary veins (record 355). She showed no gastro-intestinal symptoms except for loose stools for 5 days at the

end of her stay in hospital which lasted for 3½ months. She had frequent episodes of upper respiratory infection, however, for which antibiotic therapy was given owing to her cardiac incompensation. As seen from Fig. 2, a *Staph. aur.* strain reacting with phage 819 and resistant to antibiotics of the tetracycline type was recovered from the stools immediately after a period of terramycin treatment. During a period with no antibiotic treatment, this strain was replaced by another strain reacting with additional phages, and this second strain remained in the gut for a considerable time. Before a second course of treatment with terramycin no *Staph. aur.* could be isolated but after treatment a strain reacting with no phages and different sensitivity pattern was recovered. After this course of treatment the child had frequent loose stools.

The second case, also shown in Fig. 2, is that of a newborn boy with a severe cleft lip and palate (record 4/55). This boy developed no hospital infections, but was given oxytetracycline prophylactically for 4 days before operation. Before treatment a strain

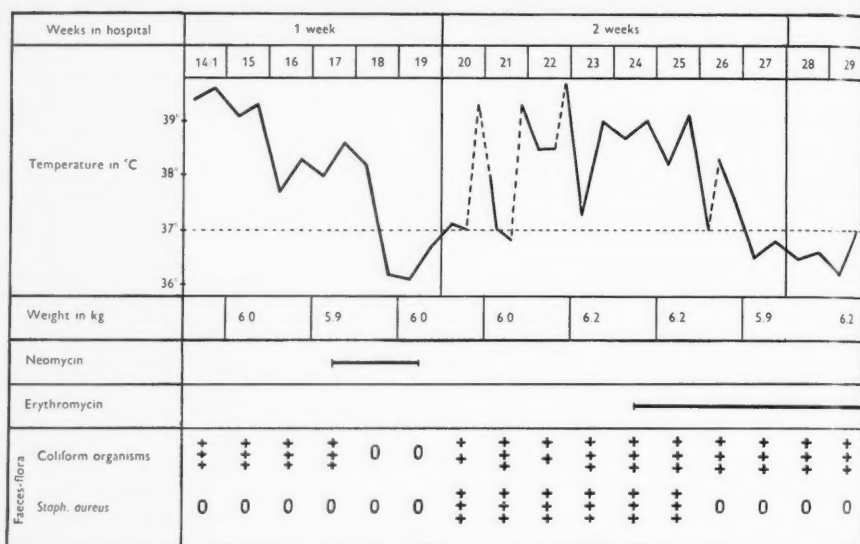


Fig. 3. A case of enteritis of probable staphylococcal aetiology following neomycin treatment.

sensitive to all antibiotics was isolated, which was untypable by phages. After this course of treatment a resistant strain was isolated which reacted with phages 166 and 155, and a similar strain was also isolated six weeks later.

These two cases further indicate that substitution of staphylococcal strains may occur in connexion with antibiotic treatment or even spontaneously. The new strains may in some cases be pathogenic as illustrated by the following case.

A 7-month-old boy with malignant ulcerative colitis (record 60/57), who 2 weeks after admission developed a hospital infection with high fever and severe diarrhoea (see Fig. 3). Neomycin was administered to suppress the intestinal flora; if a bacterial agent was responsible for these severe symptoms, improvement could be expected. Clinical improvement was in fact observed during neomycin treatment, and the temperature subsided. No staphylococci were iso-

lated during the pyretic period, but when treatment was interrupted after 3 days an abundant growth of staphylococci was obtained. Coliform organisms also reappeared. At the same time the child developed signs of enteritis, and again became very ill. The staphylococci were sensitive only to erythromycin of the antibiotics tested, and when the temperature rose for the second time this drug was administered and the temperature fell. The stools never became completely normal, owing to the colitis but definite improvement in the stools was recorded after erythromycin treatment.

### Discussion

The results suggest that treatment with antibiotics of the tetracycline type may cause substitution of staphylococcal strains in the gut by resistant strains. This is also corroborated by changes in phage-type in a number of cases. The phenomenon was observed equally often

in children with and without diarrhoea. Substitution of staphylococcal strains in the faeces during stay in hospital was also observed in children not treated with antibiotics, however, but resistance to antibiotics was less frequently observed among these new strains.

The role of staphylococci in the faeces of children is difficult to evaluate. Several reports indicate that they may be associated with gastro-enteritis or pseudomembranous enterocolitis (1, 4, 10, 11, 13), but many investigators report a high incidence of staphylococci in healthy children (6, 12). Difficulties in phage-typing staphylococci from the faeces render this technique less useful in evaluating the role of these organisms, as shown in the present study and reported previously (16). The findings in the minor epidemic described indicate, however, that the method may have its advantages. The pathogenicity of staphylococci in the gastrointestinal tract has been associated with the enterotoxin-producing capacity of the strains, tested in monkeys (14), but as long as no simpler method of testing this is available, it cannot have practical implications.

Thus the difficulty arises of how to handle the patient with staphylococci in the faeces. The results obtained suggest that antibiotic treatment with tetracyclines should not be administered prophy-

lactically to eliminate staphylococci from the gut, since this may result in substitution by resistant staphylococci of different type which may be highly pathogenic. This has also been stressed by Frisby (9). On the other hand, if diarrhoea occurs and is suspected to be of staphylococcal origin, treatment may be given. The duration of antibiotic treatment ought to be based on the clinical picture and not on elimination of staphylococci from the gut. The results further indicate, as shown by the epidemic and by one of the cases described, that staphylococci may be pathogenic in the gastrointestinal tract, and may give rise to mild or even severe enteritis. In no case was pseudomembranous enterocolitis observed. This is probably rare in children (1). A staphylococcal aetiology of the epidemic of enteritis described, and of the case of severe enteritis following neomycin treatment may be disputed because a viral aetiology was not excluded, but the results suggest at least that this was the case.

The sudden appearance of pathogenic staphylococci in the hospital thus calls for extensive study to obtain methods to distinguish between the more and less pathogenic strains. With regard to the staphylococci in faeces, a simple method for detecting enterotoxin-producing capacity is urgently needed.

### Summary

A series of children with and without diarrhoea from whose faeces *Staph. aur. pyogenes* had been recovered on some occasion was studied. Samples were taken before and after treatment with antibiotics of the tetracycline type, and, in untreated cases, at regular intervals during their stay in hospital. Phage-typing of the staphylococci was performed to some extent.

The results indicate that most children with staphylococci in the faeces had on admission a sensitive strain, which after treatment was replaced by a resistant strain the phage-pattern of which differed from that of the original strain in some cases. In some of the children not treated, the strains of staphylococci also changed after a certain period in hospital. Representative cases to illustrate these phenomena are described.

Further, a minor epidemic of enteritis, probably staphylococcal in origin, is described, and also a case of severe enteritis, which may have been caused by staphylococci after suppression of the gastro-intestinal flora by neomycin.

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## C-reactive Protein in Human Colostrum

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C-reactive protein (CRP) is generally considered to be a nonspecific indicator of inflammation or tissue destruction, whether bacterial or from other causes. Some controversy exists concerning the nature of this protein. Electrophoretic studies have revealed that the protein migrates either in the gamma globulin fraction (Wood, McCarty & Slater, 1954; Hedlund & Brattsten, 1955) or in the region between the gamma and beta globulins (Philipson & Tveterås, 1957).

The substance has been found in sera of patients, suffering from various disorders (Hedlund, 1947). The occurrence of CRP in normal pregnancy has been reported by Shetlar, Bullock, Shetlar & Payne (1955), by Rozansky & Bercovici (1956) and by Philipson & Tveterås. Rozansky & Bercovici also examined 71 maternal sera 2–12 hours before delivery. CRP was present in 66 per cent of these samples.

Although CRP might appear in the serum of the mother, a passage from mother to foetus during the intrauterine life seems unlikely to occur. Rozansky & Bercovici analysed 73 cord blood samples and found CRP in only one instance. Philipson & Tveterås examined 46 cord blood samples but failed to demonstrate CRP in any of these specimens.

On the other hand, it has been shown that CRP might be present in the sera of very young infants (Rozansky & Bercovici, Philipson & Tveterås). These results were interpreted as evidence for a production of CRP by the infants themselves.

However, the possibility remains that the substance might be transmitted from the mother to the child by way of the colostrum or milk. In order to exclude this possible route of transfer, human colostrum and milk samples were examined with regard to the occurrence of CRP, and the results of this investigation are given in the present paper.

### Methods

*CRP antiserum (CRPA) reaction.*—The reaction was carried out as a precipitation test according to Anderson & McCarty (1950). Each millimetre of precipitate was expressed as a +, the maximum degree of reaction,  $\geq 4$  mm, being + + + +.

*Löfström's reaction.*—This test was performed according to Löfström (1943) as a capsular swelling reaction of the pneumococcus type 27. Titre 1 means the lowest concentration, titre 32 the highest.

The methods are more fully described by Philipson & Tveterås.

Control experiments revealed that CRP

could be recovered in milk samples by the two tests, and no nonspecific inhibition of the reaction by the presence of milk was noticed.

### Material

Sera and colostrum or milk samples from a number of mothers, admitted to the Maternity Ward, University Hospital of Uppsala, were available. The sera were obtained on the first or the second day following delivery. A series of daily colostrum samples from the first to the fifth day post partum was secured from each mother, and in some instances cord blood samples and milk samples from the eighth day post partum were collected. The milk fat was removed by centrifugation, and all samples were handled as described by Nordbring (1957).

Twelve mothers were found to have titres of ++ or higher in the CRPA reaction and 2 or higher in the Löfström reaction in their sera. The colostrum and milk specimens from these 12 mothers were analysed.

### Results and Discussion

The results are given in the table.

TABLE 1.

	Maternal serum	Cord serum	Milk 1st to 5th day post partum	Milk 8th day post partum
No. of samples tested	—	3	60	3
No. of samples positive	12	0	0	0

It is readily seen that CRP could not be demonstrated in any of the 63 colostrum

or milk specimens analysed, although CRP was present in the sera from all 12 mothers. Most of the mothers had titres of +++ and 8 respectively in the two tests. As expected, CRP was not found in the three cord sera available.

Obviously, CRP is not secreted into the human colostrum or milk in measurable amounts, and consequently, CRP is not transferred to the infant by ingestion of colostrum or milk.

The result of this investigation seems to corroborate the assumption made in previous publications that the infant is able to produce CRP in early life. There is evidence that the formation of CRP may occur very rapidly. Rozansky & Berco-  
vici reported the presence of CRP in a baby, two days old, suffering from pemphigus neonatorum. In the one case of theirs, in which CRP was found in the cord serum, they considered the protein to be formed by the foetus itself in response to infection in utero during the protracted labour. Philipson & Tveterås demonstrated highly positive CRPA- and Löfström reactions in serum from a child, one day old, with meningitis of unknown origin.

The accumulated evidence of rapid formation of CRP, even by newborn infants, strongly supports the opinion that CRP is not of antibody nature, because the antibody formation usually requires a considerable time. The appearance of CRP in sera of very young infants suggests that the production occurs regardless of the maturity of the mechanism of antibody formation.

### Summary

C-reactive protein (CRP) was determined in colostrum and milk samples from 12 mothers with positive reactions in their sera. CRP could not be demonstrated in any of 63 colostrum or milk specimens, and a transfer of CRP from mother to child by ingestion of colostrum or milk thus is unlikely. This supports the opinion that the infant is capable of producing CRP in early life.

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## Steroid Therapy in Some Types of Post-infectious Encephalomyelitis

by GÖSTA ÖBERG

Neurological complications such as meningitis, encephalitis, and myelitis may appear after morbilli, varicella, and rubella. They are common after infectious parotitis, and may also follow infectious mononucleosis. Similar complications may also occur after immunization: the encephalitis associated with vaccination against small-pox is well known, but during the last decade it has been very rare in Sweden.

The incidence of these complications in measles, chicken pox and German measles varies in different epidemics, and an increase has been established (Hoyne & Slotkowski, 1954). This may of course largely be due to the greater facilities available, and to more exact diagnosis, even quite mild neurological signs being noted. There has probably been a true increase, but this has not been proved.

In a series of 285 cases of measles encephalitis the mortality was 20 %, 40 % of cases had residual signs, and 40 % recovered completely (Hamilton & Hanna, 1941). In Sweden the mortality from measles during the last decade has been about 0.20 per 100,000 inhabitants. In 1955 the rate was 0.29. The acute mortality rate of encephalitis after chicken pox has been about 10 %, and after rubella 20 % (Miller, Stanton & Gibbons, 1956).

No deaths from chicken pox and rubella have been reported in Sweden during recent years. The clinical course is variable, but recovery is common and after rubella sequelae are very rare. Antibiotic therapy has provided excellent weapons in the fight against bacterial complications, and we have also better knowledge about the importance of a free airway and the necessity of adequate ventilation in patients with disturbance of consciousness or dysphagia. The fatality rate must therefore now be lower than it ever has been in the past.

There are no figures for the incidence of neurological complications in these diseases in Sweden. Investigations from abroad have indicated a rate of 1–2 per 1000 (Tyler, 1957). The incidence seems to vary in different epidemics. Several investigations show that the electro-encephalogram is commonly abnormal in patients who have clinically recovered from measles encephalitis (Holmgren & Jacobsson, 1949, and Grossman & Gibbs, 1956).

Investigations in Sweden have indicated a ratio of 1:3:15 between the incidence of encephalomeningitis following German measles, chicken pox, and measles treated in hospital (Ström, 1956).

During the last 5 years we have treated some patients with severe neurological

TABLE 1.

No. Case	Age, years	Diagnosis	Main neurological signs	Treatment, days after appearance of neurological signs.	Course and sequelae
1	7	Measles	Guillain-Barré syndrome with cranial-nerve paralysis and respiratory disturbance. Symptoms appeared on the 8th day.	Tracheotomy on the 10th day after appearance of neurological signs. Respirator one week.	Hospital treatment 10 weeks. Convalescence about one year. Emotional instability, lack of concentration at school.
2	9	Measles	Guillain-Barré syndrome with paralysis of the limbs. Symptoms commenced on the 6th day.	Cortisone 2nd-4th days. (Daily dose 100-50 mg.)	Hospital treatment 2 weeks. Convalescence 2 weeks No sequelae.
3	10	Measles	Coma. Symptoms commenced on the 7th day.	Cortisone 3rd-7th days. (Daily dose 100-50 mg.)	Hospital treatment 4 weeks. Convalescence 4 weeks. No sequelae.
4	9	Chicken pox	Convulsions, Coma. Hemiplegia spastica, Papilloedema (3 dioptries). Symptoms appeared on the 7th day.	Cortisone 3rd-8th days. (Daily dose 100-50 mg.)	Six hours after beginning of treatment, fully conscious. Within one day the hemiplegia disappeared, and after two days there was no papilloedema. Hospital treatment 3 weeks. No sequelae.
5	15	Rubella (diabetes mellitus)	Coma and respiratory disturbance. Symptoms commenced on the 6th day.	Prednisone 2nd-4th days. (Daily dose 30 mg.) Immune globulin. Tracheostomy on the 4th day. Respirator three days.	Hospital treatment 3 weeks. Convalescence 6 weeks. No sequelae.
6	8	Chicken pox	On the 5th day pyrexia and headache. Admission to hosp. 6 days later. Cerebellar signs. Babinski pos. Coma.	Prednisone 11th & 12th day (20 mg/day) and 13th & 14th day (15 mg/day). Gamma-globulin.	During the first days of treatment less comatose but on the 14th day amaurosis, coma, and pyrexia (39°C). After 5 days no fever and again conscious. Hospital treatment 8 weeks. EEG still pathological after 10 months.

complications following these exanthemata. Six consecutive cases are presented in the table. The very severe course in Case 1 made it necessary to employ a

positive-pressure respirator, and the child recovered. The convalescence was very prolonged, and there was some residual disturbance.

There are many reasons for attributing the neurological signs in these states, as well as in the encephalitis following small-pox vaccination, to anaphylactic reactions. The signs appear after a period of about one week following the appearance of the rash, as in many immunological reactions. During the acute stage the patient is often very ill, with convulsions and other signs of oedema in the central nervous system. The histological picture is one of acute disseminated encephalo-myelitis with perivascular demyelination. Similar lesions of the central nervous system are found in experimental hyperergic encephalitis in sensitized animals (Ferro & Roizin, 1957).

Some steroids have a rapid and highly beneficial effect upon the hyperergic reaction. On the other hand, it is well known that corticotherapy can lower the resistance to infection (Schwartzmann *et al.*, 1953). In severe bacterial infections such as meningococcaemia, cortico-steroids as supplement to antibiotic therapy may be life-saving (Cassidy, 1957). In an infection which cannot be controlled with antibiotics, it is of course necessary to be hesitant about the use of cortico-steroids.

These neurological complications may give rise to severe, acute reactions, however, and the risk of residual symptoms is not to be disregarded. To try a short course of steroid therapy in the post-infectious phase of the illness might therefore be justified. In Cases 2, 3, and 4 the neurological signs disappeared very rapidly during steroid therapy, and the patients showed no residual signs. In Case 5 the treatment seemed to be effective on the first day, but the patient again became unconscious. On the fourth day after the

appearance of neurological signs he had to be tracheotomized, and was treated in a positive-pressure respirator for three days. This experience of course made us more doubtful about the value of steroid therapy, but some encouraging reports and in particular the investigations by Appelbaum & Abler (1956) could not be neglected. They treated 17 consecutive cases of measles encephalitis with corticotrophin. All patients survived, 15 of them recovered completely.

Further trials of steroid therapy were indicated, and a patient with chicken pox with neurological complications (somnolence and signs of cerebellar damage) was given prednisone. After two days the dose was lowered. The patient was fully conscious, and had only slight ataxia. After a further two days the temperature again increased, and the patient suddenly became unconscious. The prednisone was immediately withdrawn, and large doses of immune-globulins were administered (Case 6). After prolonged treatment and convalescence, the patient was able to return to school again, but 10 months later the electro-encephalogram is still pathological.

## Discussion

The neurological complications of these exanthemata prove fatal in 5-20% of cases. The initial symptoms may be severe, and coma can last for many days. Muscular paralysis may threaten the patient's life, and sequelae are not uncommon. Even if the incidence of severe complications is not high, follow-up investigations often show behaviour disturbances, irritability and emotional instability, lack of concen-

tration, and so on (Gibbs, Gibbs & Grossmann, 1956).

Many animal investigations have established the fact that steroids reduce the resistance to bacterial and virus infections. The depressant effect on antibody-production depends on several factors. Administration of cortico-steroids before injection of antigen causes a reduction in the circulating antibodies. The minimum dose of steroids producing this effect varies in different animal species, but the doses used in these experiments have been very high in comparison with those used in clinical therapy. In higher doses the steroids can produce a reduction in the circulating antibodies even when given after the injection of antigen (Berglund, 1956). These findings cannot be applied directly to clinical medicine. It is known, however, that a virus infection such as measles or chicken pox, or inoculation with vaccinia virus, in a person who is being treated with steroids for another condition may have a severe or even fatal course. In some of these cases, of course the primary illness (lymphosarcoma, leukaemia, or other severe blood or reticuloendothelial disease) will certainly have influenced the outcome. The danger of corticoids in virus disease is well established (Haggarty & Eley, 1956).

The situation is different if we try to make use of the antipyretic and anti-inflammatory effects of steroids during a phase of the virus disease when antibody production is probably not liable to be influenced (especially when clinical doses are employed). There is therefore probably no absolute contra-indication to symptomatic treatment with steroids in virus infections.

There are several theories about the aetiology of these neurological complications. The clinical and histological and experimental findings often support the opinion that the phenomenon is an anaphylactic reaction, although there may sometimes be a true encephalitis with primary lesions of the nerve cells.

It is striking that in one case of vaccinia gangraenosa with no capacity for antibody-production there was total absence of lesions in the nervous system in spite of massive dissemination of vaccinia virus throughout the whole body (Öberg, Nathorst-Windahl & Wesslén, 1958). In the encephalitis which may follow vaccination against small-pox, on the other hand, the lesions are similar to those seen in experimental anaphylactic encephalopathy.

The use of steroid therapy in these states is based on the concept that the clinical signs are manifestations of allergic reactions in the nervous tissue. An intense anaphylactic reaction with local circulatory disturbances may damage the nerve cells, and rapid suppression of this response is therefore desirable.

In Cases 5 and 6 there was slight improvement during the first days of treatment with prednisone. In spite of continued therapy the patients became worse again. One (Case 5) was vomiting, which could of course explain the lack of effect of oral therapy. In Case 6 the patient became worse when the dose of prednisone was reduced. Whatever the reason for the failure of steroid therapy in these two cases, it is important to report even failure. It is difficult to judge the value of therapy in an illness with a spontaneously variable course. Only prolonged experience can determine the efficacy of the treatment.

### Summary

The neurological complications of the contagious exanthemata may be very severe and dangerous. Residual symptoms are not uncommon. Even transitory anoxemia can cause cerebral lesions. Treatment by tracheotomy and respirator is sometimes life-saving. There is much evidence in support of the opinion that neurological signs following measles, chicken pox, and rubella are of anaphylactic origin. Attempts at steroid therapy may be justified. Treatment should be applied only for a few days, and then only during a stage of the illness when good antibody production can be counted on. The clinical course in three cases successfully treated with cortisone and in two cases treated with prednisone without improvement are described. Until we know more about this therapy it should be reserved for severe cases with signs of cerebral oedema or cranial nerve paralysis.

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## Pneumoencephalography in Early Infancy —Risks, Clinical Indications and Technical Considerations

by BENGT HAGBERG, ARNE HAMFELT,  
MARTIN H:SON HOLMDAHL and HERMAN LODIN

There are evidences in the literature that pneumoencephalography in early infancy may be complicated with more severe untoward reactions than in later years (1, 4, 11, 12). Even deaths have been reported. In a series of 151 encephalographies on children, e.g. Charash & Dunning (4) had two deaths and nine non-fatal but serious complications. The fatal cases were 5 and 9 months old respectively.

With the encephalographic technique used in our hospital during the years 1951-56 we observed reactions in infants, severe enough to lead to a re-evaluation of our clinical indications and to some modifications of our technique. The purpose of this paper is to present the frequency and type of complications in this earlier material, to discuss the clinical indications for encephalography in early infancy, and to report the techniques now successfully used in our hands.

### Material and Technique 1951-56

The material comprises 123 encephalograms made during the years 1951-56 in 122 infants and children less than 15 years of age. All cases less than two years of age

were analyzed separately with special references to the clinical diagnosis and the indication for encephalography. This series comprises 35 cases. The encephalographic technique used was as follows.

In the older, quiet children the examination was carried out under slight sedation with a small dose of morphine-scopolamine. In anxious children and in all children below the age of eight years, general anaesthesia was used. All these were premedicated with atropine in the usual dosage according to age and weight and sometimes Narkotal® (an ultrashort acting barbiturate) was used rectally as a basal narcosis. In most cases anaesthesia was induced with nitrous oxide-oxygen-ether and an oral endo-tracheal tube inserted when stage III, plane 3 (III:3) (ref. 6), was reached. In some of the later cases intubation was carried out after intravenous induction with Narkotal® and succinyleholine. After induction a light level of anaesthesia (III:1) was maintained during the encephalographic procedure with nitrous oxide-oxygen-ether using the Ayre's T-piece technique for children under three to four years and a semi-closed system for those above this age.

The roentgenological method routinely used was the one of fractionated encephalography (7), the ventricular system being filled with air by the lumbar route. Usually in children less than two years old 15-20 ml

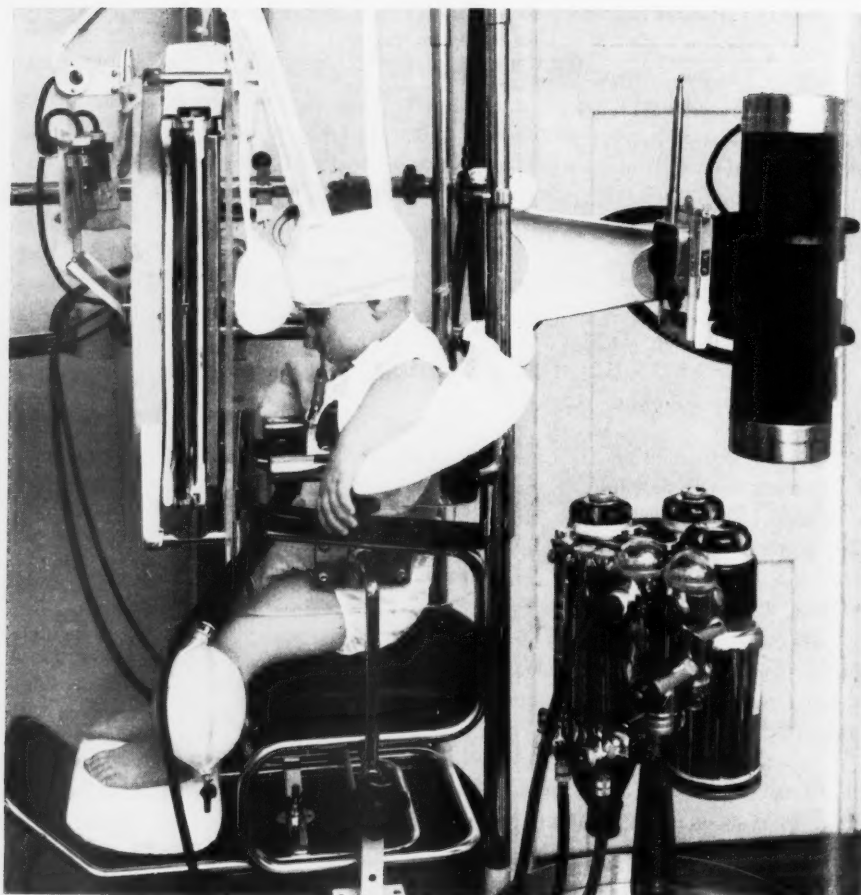


Fig. 1. Anaesthetized 2-year-old child positioned in the special chair for pneumoencephalography.

of air were injected and 10–15 ml of spinal fluid removed. The practical performance of the examination varied slightly depending on whether general anaesthesia was used or not.

If general anaesthesia was not necessary, i.e. in older children, the technique used in adults was performed. Infants and small children requiring general anaesthesia were placed in a chair specially constructed<sup>1</sup> for encephalographic examinations in this age

group. This allows the patient to be securely fastened in a sitting position (Fig. 1). A special device makes it possible to place the head in a suitable position during injection of the air by the lumbar route. When the posterior fossa including the cisterns has been investigated and the ventricular system has been adequately filled with air, the patient is laid down. The examination is then continued in the horizontal position. If the patient is too big for this special chair, but

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general anaesthesia is necessary, he is placed in the chair used for adults, where the head is held in a temporary device, which allows the position of the head to be altered as required. In all cases reported here the whole ventricular system and subarachnoidal space including the cisterns were fully examined. The child is in the sitting position for half to one hour and the whole encephalographic examination takes one to two hours.

### Results and Discussion

The frequency of the untoward reactions and their distribution in different age groups are shown in Table 1. "Slight reac-

TABLE 1. *Reactions to pneumoencephalography in different age groups.*

Age (yrs.)	No.	Reactions	
		Severe	Slight
0-1	23	4	9
1-2	13	1	3
2-4	14	1	7
4-6	12	0	6
6-8	14	0	7
8-10	17	0	11
10-15	30	0	16
Total	123	6	59

tions" are defined as slight or moderate fever for a few days, nausea, headache, and/or slight meningeal irritation. They were present in nearly half of our cases, but seemed to occur less frequently in young infants than in the older age group. The severe reactions, all of which are described below, were significantly more common in the first year of life. Life threatening situations occurred only in the younger age group. All cases with serious reactions are reported below.

CASE 1. (Record No. 354/53).—27-day-old boy. He had a known cerebral haemorrhage, diagnosed during his first week of life and

with old red blood cells in his CSF. Later he developed a slight cerebral palsy with left hemiparesis and speech disturbance.

Indication for encephalography was a possible expanding intracranial haematoma. A total of 5 ml of spinal fluid was removed and replaced by 7 ml of air. Within a few minutes the child developed a severe peripheral vascular failure and probable cardiac arrest. He improved after intracardiac injection of adrenaline and the usual resuscitative measures, but for some while afterwards had an irregular bradycardia. He had completely recovered the same afternoon.

CASE 2. (Record No. 1157/54).—3½-month-old girl with a probable perinatal brain damage. Later found to be severely mentally retarded. Encephalography was performed for increasingly frequent convulsions and general retardation and showed central and cortical atrophy. During the examination the girl was in a poor general condition with repeated attacks of cyanosis despite, as far as could be ascertained, a completely free airway. Afterwards she rapidly improved.

CASE 3. (Record No. 135/54).—11-month-old boy with multiple anomalies, later found to be severely mentally retarded. Encephalography to ascertain the extent of cerebral malformations showed agenesis of the corpus callosum. After the examination he was ill, pale with violent vomiting and fever. The next day he had markedly improved, and was afebrile on the third postoperative day.

CASE 4. (Record No. 105/55).—11-month-old girl with a severe cerebral palsy, later found to have a tetraplegia with variable tone and marked dyskinesia. Encephalography for the diagnosis of cerebral atrophy was performed with some difficulty, but without untoward reactions during the operation. During the next few hours, however, she developed increasingly severe respiratory stridor, culminating in respiratory arrest. Acute tracheotomy was performed. She slowly improved over the next few days. But it was not possible to remove the cannula until more than one month after the encephalography.

CASE 5. (Record No. 319/56).—14-month-old girl with multiple malformations (syndrome of Apert). Encephalography to ascertain the degree of cerebral malformations was attempted without success. Already during the first unsuccessful attempt to inject air the patient suddenly developed respiratory arrest. Irregular breathing returned as soon as she was laid down, but it was considered wiser to cancel the examination. She improved during the next few hours.

CASE 6. (Record No. 880/55).—4-year-old girl, acutely ill with vomiting, atactic gait, convulsions localized to the left, and conjugated deviation to the right, but with no signs of increased intracranial pressure. She was afebrile. Encephalography performed for the diagnosis of a right-sided expanding process showed no abnormalities. During the examination the patient was quite well, but within an hour of recovery from the anaesthetic, she suddenly became unconscious, was ashen-grey and vomited. She responded successfully to the usual resuscitative measures and ten days later she was sent home in a fairly good condition. The diagnosis was thought to be acute encephalitis.

Three of the above cases with severe reactions occurred during the encephalographic examination (cases 1, 2 and 5), three in the postoperative period (cases 3, 4 and 6). In only one of them, case 4, where difficulties in endotracheal intubation were encountered, could the complication be considered technically avoidable. Here a postoperative laryngeal oedema was the main cause of the respiratory distress. In the other cases, the sudden developing cardiovascular or breathing disturbances most likely were of a central origin, possibly unavoidable in a complete fractionated encephalography. This agrees with the experiences of de C. Coles (5) with small children. In case 5, a child with severe cerebral malformations, the upright

TABLE 2. *Main clinical diagnosis in relation to the type of brain damage.*

(35 infants and children, 0–2 yrs of age.)

Type of brain damage	Cerebral palsy	Mental retardation	Epilepsy (pure)	Hydrocephalus	Acute neurologic disorder
Perinatal	8	7	0	3	0
Postnatal	2	1	2	2	2
Malformations	0	4	0	3	0
Cerebral tumor	0	0	0	0	1
Total	10	12	2	8	3

position under general anaesthesia alone seemed to lead to respiratory and circulatory distress of central origin. In general more strict indications and sometimes a modified roentgenological and anaesthetic technique may help to reduce the incidence of the above reactions.

Table 2 shows the main clinical diagnosis of all cases less than two years of age. Only 3 out of 35 cases were acute neurological problems: one case of glioma of the pons and two with complicated forms of subdural effusions due to encephalitis and suppurative meningitis, respectively.

TABLE 3. *Clinical indications for pneumo-encephalography in relation to the type of brain damage.*

(35 infants and children, 0–2 yrs of age.)

Type of brain damage	Expanding process?	Hydrocephalus, obstruction?	Brain atrophy?	Meningitis?
Perinatal	0	3	15	0
Postnatal	4	1	4	0
Malformations	0	3	0	4
Cerebral tumor	1	0	0	0
Total	5	7	19	4

The clinical indications for encephalography are shown in Table 3. In two thirds of the material the clinical question were either brain atrophy or cerebral malformations, i.e. the indications were highly relative. All the cases less than two years old with serious untoward reactions belong to this group. In only five cases were expanding intracranial lesions suspected. In seven patients the indication was the differentiation between communicating or non-communicating hydrocephalus, and in the latter case the anatomical localization of the obstruction.

Of 31 successfully performed encephalographies 29 were abnormal, most of them with more or less diffuse central and cortical atrophies. In 5 of 35 cases the encephalographic findings led to the patient being sent to a neurosurgical clinic.

With the experience gained from this earlier material the following general principles were worked out for routine encephalographic examination in our hospital.

### Clinical Indications

Strikingly similar encephalographic changes with more or less central and cortical atrophies have been found in children with isolated mental retardation, cerebral palsy and cases of epilepsy without other neurological symptoms (10). Thus the roentgenological findings are by no means pathognomonic of any of these disorders and are seldom of real diagnostic value. Further, there is no good correlation between the degree of atrophy and the severity of the clinical picture (3, 4, 12). The prognostic value is therefore also doubtful in most cases. Moreover, the therapy is seldom changed by the encephalographic findings in these diseases, specially in early infancy.

In *mental retardation* considerable atrophy can be found. Identical atrophy may also be found in a child who is bright or even of superior intelligence (1). Only in cases where severe malformations of the brain have been revealed may the encephalograms be of some prognostic value. Therefore, there are hardly ever any indications for encephalographic examinations in mentally retarded children during their first years of life.

In *cerebral palsy* most cases are found to have pathological encephalograms (10). No information of practical interest is, however, to be gained except in very few cases prior to neurosurgical interventions e.g. hemispherectomy. However, to date this therapy does not apply to young children.

In simple idiopathic *epilepsy* encephalography serves no useful purpose (1, 4). In suspected cases of symptomatic epilepsy, especially of Jacksonian type, encephalography may be useful, e.g. in localizing cortical scars or meningeal adhesions, which can be treated surgically.

In *hydrocephalus* an early encephalography is necessary to decide whether a communicating or a non-communicating form is present. But, as will be discussed below, it is not necessary to perform a full radiographic examination in the first instance. A "bubble study" can give all the information needed in a high percentage of cases.

In conditions where an *expanding intracranial lesion* cannot be excluded a full encephalographic examination is usually required. However, cases with long-standing subdural hematomas or effusions are

better localized by a combination of subdural taps with air insufflation and/or carotic angiography.

Thus the only strong indications for encephalography in early infancy are cases with progressive hydrocephalus or where an expanding non-subdural intracranial lesion is suspected. It appears to us desirable to keep strictly to these indications in the younger age group. In older children, however, encephalography can be performed on more relative indications and may sometimes be valuable in the investigation of non-progressive neurological disorders.

### Anaesthetic Technique

When choosing an anaesthetic technique for lumbar encephalography one has to consider that the patient will be kept in a sitting position with the head flexed for a considerable time. It is therefore preferable that whenever possible the investigation should be carried out under local analgesia, i.e. when the child is quiet and old enough to keep still when roentgenological films are taken. Premedication must still be as light as possible. Morphine-scopolamine is best avoided as it causes undue central depression and lack of cooperation. We now use an oral barbiturate. Facilities for immediate intubation, should complications occur during the examination, must always be available.

If general anaesthesia must be used for reason of age, fear or neurological status, we consider premedication with only atropine advisable. Anaesthesia must be light enough to avoid vasomotor disturbances in the sitting position, but deep enough to avoid straining on the tube or vomiting (5). Intubation under ether as

practised in the early part of the series required an undesirable deep anaesthetic level, which often caused blood-pressure fall when the child was placed in the sitting position. We therefore now routinely use succinylcholine given intravenously or intramuscularly to facilitate intubation after induction with nitrous oxide-oxygen or with a small intravenous dose of Narkotal®. Ordinary rubber tubes with a "built-in curve" are avoided as they may exert undue pressure on the arythenoid area of the larynx (8) and tend to kink when the head is flexed forward. Tubes with a wire spiral incorporated throughout their length are used. The tubes are smeared with a water-soluble anaesthetic lubricant.

When the effect of succinylcholine has worn off and spontaneous breathing is fully re-established the child is placed in the sitting position. The anaesthetic level is maintained as light as possible with 50 % nitrous oxide-oxygen and a small dose of ether, using in children under the age of three a modified Ayre's T-piece technique with a thin-walled, double-ended rubber bag at the end of the exhaust tube of the Ayre's T-piece (9). This allows for rapid control of respiration should it become necessary. In older children a unidirectional semi-open system is used.

With the modern spark-proof roentgenological equipment at our disposal we consider the risk of introducing ether smaller than giving too high a concentration of nitrous oxide. In the last few cases we have substituted ether for the non-explosive anaesthetic Fluothane® in a concentration of 0.5 % with very good results.

In the postoperative period the child is kept under very close observation in order

to detect early signs of raised intracranial pressure or airway obstruction.

### Roentgenologic Technique

All older children not requiring general anaesthesia as well as infants and small children with suspected expanding intracranial lesion are fully investigated by means of a fractionated encephalography (patients with choked discs of more than 2 D. have not been examined, as there are no neurosurgeons available in our hospital).

In babies with hydrocephalus, a so-called bubble study is performed. Without general anaesthesia a lumbar puncture is made and 5 ml of air is injected while the baby is held in a suitable upright position. The needle is withdrawn, the baby laid down and the first picture taken. If air has entered the ventricular system, the proved communicating hydrocephalus is then out-

lined so as to demonstrate the degree of cerebral atrophy. If no air is found in the ventricles either an obstruction is localized, e. g. a stenosis of the aqueduct, or we have to proceed with a fuller examination using the fractionated method under general anaesthesia or ventriculography.

In all other cases in the younger age group, where the clinical picture shows no posterior fossa lesion and where encephalographic examination may be considered of value, general anaesthesia combined with a modified roentgenological technique is used. Initially the patient is placed in an upright position to control that air is entering the ventricular system. This is then filled without any further radiological control, and the examination is immediately carried out in the horizontal position. Thus the time in erect position has been considerably reduced to the benefit of the anaesthetized infant.

### Summary

In a series of 123 encephalographic examinations performed on infants and children during the years 1951-56, serious reactions were found only in the youngest age group, four out of six being less than one year old. The probable causes of these reactions are discussed. From the experience gained in this series the clinical indications and a scheme of techniques for encephalographic examination in different cases and age groups are outlined.

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## Physical Symptoms and Psychogenic Etiology

### An Investigation of Consultation Material

by INGVAR NYLANDER

In a hospital with different departments a preliminary sorting out of the patients is carried out before admittance. Some cases can be classified at once as internal-medical, surgical or psychiatric, often even by the relatives themselves. They are then admitted to their appropriate departments. Sometimes, however, it may be difficult to form a diagnosis even after the patient has been admitted to the hospital, as the case may prove to be more complicated than was at first suspected. This occurs when unknown etiological factors, either dependent or independent of one another, appear. It also occurs where the symptomatology is diffuse or misleading.

In this type of situation consultation work plays an important part in the forming of a clinical analysis.

The examination of these cases in a children's hospital is often a very difficult task for the child psychiatrist. He is often obliged to restrict himself to an extremely limited line of questioning which is frequently a matter of guesswork. In complicated cases he must weigh in his mind the significance of hard-to-interpret or indistinct clinical findings. These con-

ditions often prevail within the field of psychiatry. The psychiatric diagnosis requires knowledge of the personality of the patient as a whole, i.e. his physical and mental health, his environment, the amount of strain and stress to which he is exposed as well as his way of reacting to it in differing situations. To gain a true picture from the psychiatric point of view of a patient, usually physically ill, who is daily being subjected to the strain of hospital environment, a variety of examinations is sometimes well-nigh impossible.

The conclusions drawn by the psychiatrist from the results of his investigations in this sphere are often quite different from those which a purely psychological interpretation of the case might lead one to expect. His judgment of a case must be based on a highly specialized knowledge and experience of clinical work. Mental symptoms, anxiety for example, need not necessarily be psychogenic but may instead be a symptom, possibly even the first, of a serious physical disease.

In order to be able to form a true judgment of different situations close collabo-

ration between psychiatrist and pediatrician is essential. Without such collaboration the psychiatric diagnosis becomes merely descriptive and the clinical evaluation cannot progress.

The object of this essay is to give an account of the results of an investigation carried out with material obtained from the Outpatients' Department of Psychiatry, Kronprinsessan Lovisas Children's Hospital, Stockholm, and to present certain reflections in connection with this. The physical investigation of the cases has been carried out by the hospital's medical and surgical departments, often parallel with the psychiatric examination and in consultation with the psychiatrist. In not a few cases the final diagnosis was the result of this co-operation.

### Case Material

The case material is comprised of all the cases which were referred to the outpatients department for consultation from medical and surgical departments during the period 1st January, 1954 to 30th June, 1957, 465 patients in all, of which 228 were boys and 237 girls.

TABLE 1. *Yearly distribution of cases.*

	1954	1955	1956	1/1-1/7 1957	Total
Boys	59	56	76	37	228
Girls	54	64	67	52	237
Total	113	120	143	89	465

The distribution of the series over the different calendar years is shown in Table 1. It will be noticed that the number of consultation cases has increased by approximately 65% from 1954-1957.

The ages of the children are, roughly speaking, evenly divided among the group 1-14 inclusive.

The differences in social background have been taken into account and the material is classified according to the principles of official Swedish statistics. The children are classified according to the occupation of their parents. The social-group distribution agrees in the main with that drawn up in the 1948 yearly election statistic.

Unmistakable somatic disease or somatic signs and symptoms were found in 263 cases or 57% of the whole series, (130 boys, 133 girls), see Table 2. Epileptic seizures of different kinds occurred in 85 cases (42 boys, 43 girls) or 18% of the whole series. Other types of symptoms connected with the central nervous system appeared in a total of 58 cases (41 boys, 17 girls) or 12% of the whole series. The majority of these cases consisted of cerebral pareses. In isolated cases hydrocephalus, brain tumor, head injuries, etc. were found. Endocrine disturbances were detected in 51 cases or 11% of the whole series (21 boys, 30 girls). The remaining 69 patients (26 boys, 43 girls) were found to be suffering from the conditions shown on Table 2.

Greatly retarded development was constituted in 37 cases (19 boys, 18 girls). Of

TABLE 2. *Somatic diseases in the material.*

Type of illness	♂	♀	No. of cases	Frequency percentage in whole material (465 patients)
Epileptic seizures	43	42	85	18
Other neurological diseases	39	19	58	12
Endocrine disturbances	20	31	51	11
Infectious diseases	5	6	11	16
Heart diseases	5	5	10	
Malformations	2	6	8	
Surgical diseases	1	5	6	
Peptic ulcers	1	3	4	
Allergic diseases	2	2	4	5
Intoxications	1	1	2	
Miscellaneous	11	13	24	5
Total	130	133	263	57

these 11 were given the diagnosis mongolism (7 boys, 4 girls).

Somatic disease was not found in 202 cases or 43 % of the total number (98 boys, 104 girls).

### Methods

The majority of cases have been examined both by psychiatrists and psychologists. Usual psychological investigation methods were used in this connection. Intelligence and development examinations were carried out according to the methods of Terman-Merrill and/or Bühler-Hetzer and in isolated cases according to Grace-Athur and Gesell, or Gesell only. Information about the parents' professions, civil status (married, unmarried or divorced) and number of children were obtained regularly from the census office in Stockholm. In those cases where there was reason to suspect that environmental conditions had an important bearing on the patient's symptoms socio-medical enquiries were carried out through a social worker and information was collected from schools, day-homes, etc. In all other cases the social particulars shown in the patients' records from the somatic department were accepted as being adequate.

### Results

The following paragraphs give a description of the results achieved through the psychiatric-psychological examination. It would, of course, have been desirable to weigh the examination results against the indications which rendered the consultation necessary. This, however, has not been possible as the indications have often been difficult to define exactly. Two doctors (the head of psychiatric department, Prof. S. Ahnsjö, and the author) have been responsible for the examination of over 90 % of the cases, their examination methods and standards of judging being similar. In an analysis of the series by division into groups according to calendar

year, no differences were found with regard to sex, age, social group, frequency of mentally ill parents, broken homes, birth injuries, head injuries, etc., and for this reason the series will from now on be treated as a whole.

#### *No noticeable mental symptoms*

In 89 of the 465 cases or 19 % of the whole series no noticeable mental symptoms were found (44 boys, 45 girls).

Of these, 79 (40 boys, 39 girls) had a somatic disease of one of the above-mentioned types. In rather more than half of these cases a neurological condition was present and in about a quarter of the cases endocrine disturbances were found. In one case the child was submitted for examination on account of insanity in the mother. Adoption enquiries (i.e. health examination of child prior to adoption) occurred in 3 cases.

#### *Retarded development*

Both cases achieving, when tested, an I.Q. of under 85 and those who, with or without I.Q. tests, were judged as needing institution care have been classified as intellectually retarded in development.

Thus 164 cases were placed in this category (87 boys, 77 girls), that is, 35 % of the whole series.

Of these 58 (28 boys, 30 girls), that is, 35 % were classified as feeble-minded (I.Q. usually 70-85). The majority (41 cases) were found to be suffering from somatic diseases, usually of a neurological type, or endocrine disturbances. The origin could be traced in 43 % of the cases.

Sixty-four (32 boys, 32 girls) or 39 % with I.Q. varying between 35 and 70 were classified as imbeciles, but teachable.

Forty of these had somatic disease and the origin could be traced in 53 % of the cases.

Imbecilic to the extent of being unteachable (I.Q. under 35/50) was the classification given to 42 cases (27 boys, 15 girls), i.e. 26 % of those with retarded development. Twenty-two of these had somatic disease. The origin could be traced in 44 % of cases.

#### *Mental symptoms combined with normal intelligence*

Mental symptoms without retarded development occurred in 212 cases or 46 % of the whole series. In these cases somatic disease of one or other of the types mentioned above was found in 81 cases (35 boys, 46 girls) while mental symptoms of this type without simultaneous somatic disease were present in 131 of the 465 cases (62 boys, 69 girls), i.e. in 28 % of the whole series.

#### *Symptom groups*

The mental symptoms have been roughly divided, as follows, into two groups. In the first group, "uncomplicated symptoms", have been placed such symptoms as irritability, emotional instability, restlessness, difficulty in concentrating, mental depression and so on, and also symptoms of the type enuresis, enkopresis where these have had a definite psychic origin and thus not been caused by physical illness. In the second group, "conversion symptoms", have been placed umbilical colic, headaches and giddiness, astasia-abasia, joint and heart troubles and so on, that is to say, symptoms which do not, at first glance, appear to be of a definite psychic nature. Conversion symptoms are shown in Table 3.

TABLE 3. "Conversion" symptoms in patients of normal intellectual development without somatic disease.

Type	No. of cases		
	♂	♀	Total
Digestive tract symptoms	9	16	25
Headache, often combined with giddiness	10	9	19
Symptoms from joints and extremities	2	5	7
Cardiac symptoms	3	4	7
Astasia-abasia	0	3	3
Headache plus abdominal pains	1	2	3
Disabling tiredness	0	2	2
Tics	0	2	2
Ocular symptoms	1	1	2
Total	26	44	70

If we study the occurrence of these two symptom types throughout the series we notice that, while the "uncomplicated mental symptoms" are evenly distributed among the different age groups, the "conversion symptoms" appear almost exclusively among children of school age or older (Fig. 1).

The age factor is thus seen to play a decisive part in the final formation of the symptomatology and it is, in fact, well

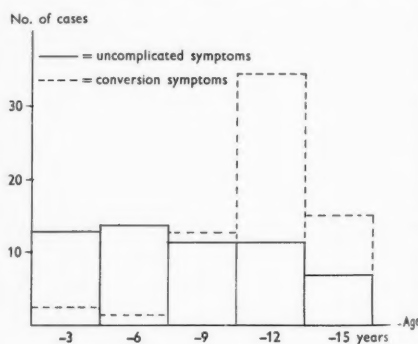


Fig. 1. Age distribution among patients of normal intelligence with uncomplicated and conversion symptoms respectively.

TABLE 4. Results of psychiatric examination of patients with normal intelligence.

Somatic condition	Sex	Mental symptoms						Diff. 0 – K	Total
		None		Uncomplicated = 0		Conversion = K			
		Freq.	%	Freq.	%	Freq.	%		
With symptoms	♂	40	53 ± 5.8	32	43 ± 5.7	3	4 ± 2.3	39 ± 6.1	75
	♀	39	46 ± 5.3	42	49 ± 5.3	4	5 ± 2.4	44 ± 5.8	85
	♂ + ♀	79	49 ± 4.0	74	46 ± 4.0	7	4 ± 4.8	42 ± 6.3	160
Without symptoms	♂	4	6 ± 9.2	36	54 ± 6.1	26	40 ± 6.0	14 ± 8.6	66
	♀	6	8 ± 9.8	25	33 ± 5.3	44	58 ± 3.6	– 25 ± 6.5	75
	♂ + ♀	10	7 ± 2.4	61	43 ± 4.8	80	50 ± 5.2	– 7 ± 7.2	141

known in the child psychiatrist's experience that environmental injuries produce more general symptoms among younger children, whereas more manifest psychosomatic symptoms tend to develop at a later age. This seems to be particularly noticeable in connection with graver forms of environmental injury where more obvious psychosomatic symptoms may be found even in younger children.

An important question is this: what significance has somatic disease in connection with the appearance of mental symptoms or, in other words, which mental symptoms appear in connection with somatic disease? In order to find the answer to this question children with normal intelligence suffering from a somatic disease were compared with children of normal intelligence without somatic disease.

It was then found that the mental symptom picture differed in the two groups (see Table 4). In the group with somatic disease either "no mental symptoms" or "uncomplicated symptoms" appeared, the latter in a statistically confirmed higher frequency than "conversion symptoms" (diff.  $42 \pm 6.3\%$ ). The difference applied both to boys (diff.  $39 \pm 6.1\%$ ) and girls ( $44 \pm 5.8\%$ ). The frequency of "con-

version symptoms" were here remarkably low ( $4-5\%$ ). This difference between "uncomplicated symptoms" and "conversion symptoms" within the group with somatic disease cannot be explained by age differences within these two groups, as, in the first place, it applies to both sexes and, in the second, the girls are as fully represented from preschool age as from the school-age group. The boys, on the other hand, are mostly of school age, so that more "conversion symptoms" could be expected (see Table 5).

In the group without somatic disease we do not find any statistically confirmed difference between the frequency of "uncomplicated symptoms" and "conversion symptoms" where the boys are concerned, though a high frequency of "conversion symptoms" is apparent ( $40 \pm 6.0\%$ ). We find, on the other hand, a statistically confirmed higher frequency of "conversion symptoms" than "uncomplicated symptoms" where the girls are concerned (diff.  $25 \pm 6.5\%$ ).

The above shows that the mental symptom picture in physically ill patients really differs from that of patients who are physically well. It is, at the same time, possible that mental symptoms of the con-

TABLE 5. *Distribution of normally intelligent among different age groups.*

Somatic condition	Sex	Age				Diff. $f-s$	Total
		-6 years = $f$		7- years = $s$			
		No.	%	No.	%		
With symptoms	♂	22	29 ± 5.5	53	71 ± 5.5	-42 ± 7.8	75
	♀	39	46 ± 5.3	46	54 ± 5.3	-8 ± 7.6	85
	♂ + ♀	61	38 ± 4.9	99	62 ± 4.9	-24 ± 6.9	160
Without symptoms	♂	19	29 ± 5.6	47	71 ± 5.6	-42 ± 7.9	66
	♀	20	27 ± 4.7	55	73 ± 4.7	-44 ± 6.7	75
	♂ + ♀	39	28 ± 3.8	102	72 ± 3.8	-44 ± 5.3	141

version type tend to be overlooked where the physically ill patient is concerned. If this is so, it would seem that certain symptoms of somatic disease are actually of psychic origin.

If it is the somatic disease in itself which causes mental symptoms and if, furthermore, these symptoms are of a different nature from those of the physically well patient, one would expect differences in the case history between cases with somatic disease and mental symptoms, on the one hand, and cases without somatic disease, but with mental symptoms, on the other. Trying conditions such as men-

tal illness of the parents, broken homes and so on, should, if this is so, exist in a lesser degree in the case of the physically ill patient than the physically well patient. This is, in fact, the case (see Table 4).

Organic brain injury is considered by many to be a common cause of mental symptoms. It seemed, therefore, to be of relevant interest to study the conditions in connection with the material. The cases chosen for this study were those with a normal I.Q. who suffered from epileptic seizures. A relatively homogeneous group was obtained. It included those cases which showed signs of cerebral irritation.

TABLE 6. *Mentally ill parents and "broken homes" among normally intelligent.*

Home environment	Sex	Normally intelligent				Diff. $M = N$
		With somatic disease = $M$		Without somatic disease = $N$		
		Freq.	%	Freq.	%	
Mentally ill father	♂	8	11 ± 3.6	22	33 ± 5.8	-22 ± 6.8
	♀	11	13 ± 3.6	22	28 ± 5.2	-15 ± 6.3
	♂ + ♀	19	12 ± 2.6	43	30 ± 3.9	-18 ± 4.6
Mentally ill mother	♂	8	11 ± 3.6	13	20 ± 4.8	-9 ± 6.1
	♀	10	12 ± 3.4	22	29 ± 5.0	-17 ± 6.1
	♂ + ♀	18	11 ± 2.5	35	25 ± 3.6	-14 ± 4.4
"Broken homes"	♂	12	16 ± 4.1	28	42 ± 6.1	-26 ± 7.4
	♀	13	15 ± 3.9	28	38 ± 4.1	-23 ± 5.6
	♂ + ♀	25	16 ± 2.9	56	40 ± 4.1	-24 ± 5.0

TABLE 7. *Some case history data plus symptom picture among patients with grave hysterical traits.*

G = girl; B = boy; Psych. = mentally ill; Alc. = abuse of alcohol; D = divorce.

Case No.	Sex	Age	Father	Mother	Home conditions	Earlier mental health	Actual symptom picture
26/54	G	7	Psych.	Psych.	D	0	Attacks (breathholding spells of hysteria).
56/54	G	9			D	0	Rumination.
301/54	G	11			D	0	Hysterical paralysis.
9/55	G	10	Alc.		D	0	Disabling tiredness. Bedridden.
189/55	G	13	Psych.	Dead		0	Attacks (breathholding spells of hysteria).
237/55	G	11		Psych.		0	Hysterical paralysis.
343/55	B	12				0	Hysterical paralysis.
475/55	G	13			D	0	Attacks (breathholding spells of hysteria).
487/55	G	10		Psych.		0	Limping walk.
490/55	G	12	Alc.	Psych.	D	0	Limping walk.
59/56	G	13	Alc.		D	0	Disabling tiredness. Bedridden.
66/56	B	13	Psych.		D	0	Disabling tiredness. Bedridden.
90/56	G	10	Alc.	Psych.		0	Astasia-abasia.
165/56	G	13	Alc.			0	Disabling tiredness. Bedridden.
201/56	G	12			D	0	Astasia-abasia.
404/56	B	13		Psych.		0	Simulation.
124/57	B	13		Psych.	D	0	Simulation.
172/57	G	13			D	0	Limping walk.

The group thus consisted of 44 cases (23 boys, 21 girls). An analysis proved that these cases in no way differed in their symptomatology from the rest of the cases with somatic disease.

The above speaks for the fact that mental symptoms are often found in connection with somatic diseases and that these are in the majority of cases of the "uncomplicated" type, i.e. of a different character than those occurring in connection with the physically well.

Of particular interest in the group which comprises cases of normal intelligence with mental symptoms and no somatic disease was a group of 18 cases (4 boys, 14 girls) with a strong hysterically colored symptom picture of a psychoso-

matic nature. These cases are described in Table 7.

They concern, in the majority of cases, girls who have reached puberty or the beginnings of puberty, who had not earlier shown any particular mental symptoms, but now showed strong psychosomatic symptoms. Where these cases were concerned almost every child had lived under extremely trying home conditions, and in most cases with a brutal or sadistic father. It seems most likely that the home environment has in these cases been the deciding factor in the development of these grave symptoms.

An example of the way in which a grave environmental situation may affect the symptom picture in a particular manner is

seen in a group of children where the father was found to abuse alcohol. This group consisted of 32 cases (13 boys, 19 girls). Of these, four were free of symptoms (12 %), eleven had "uncomplicated" symptoms (35 %) and the remaining seventeen (53 %) had "conversion" symptoms. A check-up of the cases with uncomplicated symptoms showed that five cases were children under school age with enkopresis. It may be mentioned that among children under school age with somatic disease and mental symptoms enkopresis occurred in one out of a total of 28 cases.

We see that children of fathers who were found to abuse alcohol often had mental symptoms, children below school age, enkopresis, and school age children, "conversion" symptoms. The author intends, by means of a widely extended examination to make a closer study of the symptom-picture development in children of alcoholic parents.

### Summary

The difficulties accompanying psychiatric consultation work are discussed. Case material of this type is of particular interest in that the psychic nature of the patient's condition is often not immediately apparent, but only appears in the course of a closer analysis of the case. Results are given of a synthesis and analysis of a psychiatric examination of 465 so-called "consultation cases" (228 boys and 237 girls) from the medical and surgical departments of Kronprinsessan Lovisas Children's Hospital, which was carried out during the years 1954-1957.

Somatic disease or somatic signs and symptoms occurred in 57 % of the total number of cases. In about half of these neurological diseases were found, in a quarter, endocrine disturbances. The remainder consisted of cases who had been referred here simply on the grounds of suspected psychic disturbances.

The psychiatric investigation showed that in 19 % of the cases registered, no particular mental symptoms could be found. The majority of these showed physical signs and symptoms and the remainder were to be examined for pre-adoption purposes or something similar. In 35 % of cases intellectually retarded development of varying types and degrees was present. Mental symptoms combined with normal intelligence existed in

### Therapy

In 117 cases or 25 % of the entire material psychiatric treatment was considered to be justified. Of these 19 have been admitted to the child psychiatry department, while 98 (54 boys, 44 girls) have been recommended to their own or another outpatient department for treatment. In isolated cases other special measures have been taken simultaneously with this. In 4 cases a child welfare committee was notified on account of serious mental maltreatment and in some cases places have been found in homes for nervous and psychopathic children, in nursing homes or in foster homes. In the majority of cases it has been a question of an investigation which has shown further treatment to be unnecessary, or cases with more minor mental symptoms where specialized therapeutic treatment was not considered necessary.

46% of the series. In more than half of these cases no noticeable physical disease was found. This last group comprises 28% of the entire material.

The mental symptoms in those cases with normal intellectual ability have been roughly divided into cases with "uncomplicated" symptoms and cases with "conversion" symptoms respectively.

While the uncomplicated symptoms were found to be evenly distributed among the different age groups the conversion symptoms appeared almost exclusively among the children of school age and over.

It has become apparent through analysis that in those cases where somatic disease existed side by side with mental symptoms the latter have almost invariably consisted of uncomplicated symptoms. On the other hand, in those cases where only mental symptoms were found, the incidence of uncomplicated and conversions symptoms was found to be about equal. The reasons for this have been discussed and the difference between the groups may, among other things, possibly be accounted for by the fact that conversion symptoms tend to be more easily overlooked in patients with somatic disease. Conditions of stress in the home environment existed, however, to a lesser degree in patients with somatic disease as well as mental symptoms, than in connection with those patients who only showed mental symptoms. This speaks for the fact that the somatic disease in itself or factors connected with it play a significant part in the appearance of the mental symptoms which take the form of "uncomplicated" symptoms. It was also of interest to note that the mental-symptom picture in patients of normal intelligence who showed signs of brain injury (epileptic seizures) was no different from that of normally gifted patients with somatic disease of another type.

The mental symptoms in the physically healthy showed themselves in some cases to be of a grave hysterical character and here, almost without exception, very serious environmental conditions were found to exist.

In 25% of the total number of cases continued psychiatric treatment was considered necessary.

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## The First Smile

### A Developmental Study

by BERTIL SÖDERLING

All behaviour develops from a highly undifferentiated level. Fumbling and groping in time reveal form and functional value. The faculty of speech grows through grunts, vociferation, intonation, and word-formation from the undifferentiated sound mass. The ego is concealed behind a flummery of unintelligible expressions about the infant's own body and his surroundings.

From indefinite, wavering glances and athetotic facial expression the first deliberate smile gradually dawns. In contrast to distasteful sensations, *pleasure* is early demonstrated by the first smile. This is a highly remarkable phenomenon characterized by a co-operative, "positive" attitude, in contrast to the crying and screaming produced by unpleasant sensations.

The course of events is as follows. The infant fixates intensively on the visitor's face for some while, studying it as though to form an opinion. If approval is granted, this is shown by a smile, which starts in the expression. In a manner difficult to describe, this lights up; the eyes are the first to smile, and then comes the mimic smile that the facial musculature can pro-

duce. Just before smiling and this facial action just having started, the baby's whole personality cooperates. Disintegrated, "athetotic" reflex movements of head, limbs and trunk may be combined with undifferentiated sounds, small regurgitations, etc. The totality of the baby's personality is engaged in the first smile.

It is undoubtedly not true that this smile is the result of visual perception alone. Stimuli from other sensory organs are also recorded and can produce a smile. Hearing and touch are important in a blind infant, but are also employed in a normal seeing child. Visual perception, however, would seem to be the easiest and earliest path of association.

Some of the problems that confront everyone working on the modes of expression of the emotions also perplexed Darwin (1). He was investigating the facial expression of different emotions. Did children of all races pout when they were cross? Was fear expressed in the same manner all over the world as among Europeans? Did the child frown when it was about to cry? (Here Darwin made comparative studies in anthropoid apes.) Was the smile the product of maturity and social mimicry? Was it to be found among



Fig. 1. The girl O, aged 2 weeks.

human beings of all cultures? Was the smile a primitive, species-specific phenomenon?

Our impression is that the smile is as much a part of our *specific human* behaviour as tail-wagging is a specific expression of pleasure in the dog. There is no doubt that the smile appears at a certain definite level of development and by certain, though varying, stimuli. Until this level is reached, the child cannot smile. The phenomenon may be produced by various sensory stimuli, through seeing, touch, general feeling of well-being, pleasurable activities, contact with the mother, etc.

Like the problem of the child's mimicry of human facial expression, the first smile has attracted attention largely from the gestalt school of psychologists. Charlotte Bühler (3), Martha Guernsey (4) and Volkelt (7) have provided impressive contributions. Among nordic workers, E. Kaila (5) of Finland made a comprehensive study of 7 infants aged from 2 to 7 months. He

classified the development of mimicry as follows: observation of lip-movements, rapport through the visitor's smile, etc. This sequence is followed by spontaneous "innervation" of the child's own mouth, whereupon spontaneous mouth movements take place in a similar manner to other "social" mouth movements. Later, a tendency to mimicry may be observed. These "social" mouth movements are analogous to the sucking movements which the very young infant makes on social contact, and which are a totally undifferentiated phenomenon, and may be evoked by a variety of stimuli.

Kaila's theory seems very probable. The muscles about the mouth respond to a great variety of stimuli, first by sucking movements, and later by smiling (which may also be evoked by certain pleasurable stimuli). All development starts from a highly undifferentiated level, and passes through the primitive reflex stage to conscious cerebral cortical activity, when strong stimuli or marked affect are re-

quired to arouse the responses which were formerly primitive and reflex. Just as the infant gradually learns deliberately to "save up" the sucking movements for clearly differentiated purposes, such as to imbibe food, the smile gradually acquires the character of a closely defined social response. From now on the process of learning to imitate is in progress. We all recognize the more sophisticated attitude of the 5-month-old baby to the visitor, who is subjected to a prolonged "investigation" and is not always rewarded with a smile; whereas the 2-month-old baby, for example, is far less critical.

The view held by Bühler and others, that the child smiles only into a human face is clearly untrue. Blind children smile; and seeing children often smile and even laugh aloud for joy, either at play or from general satisfaction, such as after a meal.

The above-named researchers do not give such detailed information about the age at which the first smile appears as they do about its character. Every textbook on child psychology and paediatrics, and all popular works on the subjects contain categorical statements to the effect that *the infant produces his first smile during the end of the first quarter*.

Since I could not quite agree with this view, and since *the first smile would seem to be a very important milestone in psychological development*, I have examined the problem.

When does the normal child produce his first smile, and what is it that conditions the event? Is this expressive phenomenon associated with the degree of maturity alone, or does imitation learning also play a part?

No statistical investigations into the

appearance of the first smile under different environmental conditions seem to exist. My very definite impression is that infants that have had little personal attention and those that are emotionally unsatisfied (hunger, deprivation, etc.) do in fact tend to smile later. It seems entirely probable that these factors also have a social aspect in mental development. The date of the first definite smile is probably a product of maturity and the effect of environment, the latter being a very minor multiplier compared with the former as multiplicand.

The investigation which is now presented indicates maturity to be the chief factor.

Over a period of several years the first smile has been recorded in cases from the infants' wards, child welfare centres, my private practice, and infants' homes, all the observers, doctors and nurses having been well trained in psychology. Strict conditions were set up: a true smile, starting with searching of the observer's face, the infant's eyes then starting to make contact, and finally the typical, brightening, happy look that precedes the expressive muscular movements of the face that we call smiling. All indefinite expressions were excluded; and occasional, unreproducible grins were, of course, not counted, even though they resembled smiles. On ward rounds I have tried to sustain the interest of the staff in these principles.

The photograph illustrates a typical smile in O., a girl aged 2 weeks.

In a series of more than 400 infant of normal weight at birth, and probably neither pre- nor post-mature, the first smile was recorded as shown in the following table:

*No smile*

Before 2 weeks  
(but several infants seemed to be fixating  
on the observer's face)

*Smile for the first time*

11 % between 2 and 3 weeks  
49 % between 3 and 4 weeks  
21 % between 4 and 5 weeks  
19 % between 5 and 6 weeks

Thus all smiled before 6 weeks of age, and more than 60 % at or about 4 weeks. Most were found to be mature enough at 2½-4 weeks to produce a smile conforming to the conditions laid down.

Investigations are proceeding to establish the date of the first smile of the premature and postmature infant.

*O.*, a boy, was born on February 1st, 1957, and weighed 4270 g. He was calculated to be about 4 weeks "late". His mother, who is a social worker, tained in child psychology, sent me the photograph reproduced above, and stated that the child produced his first smile at the age of 2 weeks. The photograph was taken on February 15th, 1957.

*Monika K.* (Record No. 896/56) was born on August 8th, 1956, and had a birth weight of 1600 g. We stated her level of psychological development at "full-term" level (6, 7). She laughed on September 17th, viz. at about 5 weeks, as I was able to confirm personally.

*Perti K.* (Record No. 876/56), a boy, was born on July 31st, 1956, and weighed only 1040 g. He was very feeble, and psychologically extremely premature. It is of interest to note that the child's first smile took place at about the stage when he fulfilled the conditions of full-term psychological maturity. A laugh was confirmed at about 10 weeks (October 13th). Even in this case of

marked prematurity, the smile appeared not later than 2½ months after birth.

*B.* (Record No. 1315/56), a boy, weighed at birth 1200 g, and was strikingly premature. He was born about 2½ months before the expected date. He survived the early period of feebleness, developed satisfactorily, and showed no signs of organic disease. His first confirmed smile did not take place until the age of 3 months, when he weighed 3480 g. He continued to develop into an apparently intellectually normal child.

In summary, the following may be said.

The smile and the laugh are specific human forms of reaction and behaviour. The date of the child's first smile depends very largely on the degree of maturity and psychological development. To what extent the phenomenon is conditioned by learning and mimicry, that is to say, the influence of social environment, is undetermined. Healthy, "full-term" infants are found to produce their first smile between 2 and 6 weeks, never earlier or later. The age of 3-4 weeks is evidently the average for the appearance of the first smile. Postmature infants seem to be earlier, and truly premature infants considerably later: investigations are proceeding. Within the latter category may be found a number of examples illustrating the close relationship between the date of the first smile and the degree of psychological maturity; and the child's skill in fixating and smiling would seem to be valuable late evidence in support of our theories (6, 2) concerning psychological maturation in newborns with low weights at birth.

### Summary

In a series of 400 healthy infants of normal weight at birth 70 % smiled between 3 and 5 weeks, none before 2, all before 6 weeks. The date of the first smile would seem to give correctly the developmental stage. The origin of the smile is discussed.

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## Treatment of the Obese Child

by ARVID WALLGREN

Obesity is practically always produced by an excessive caloric intake, generally combined with an inadequate energy output. The logical treatment should, therefore, consist of decreased calories and increased exercise. However, this formulation may be carrying simplification a bit too far. Such treatment is usually only symptomatic and not causal, because it fails to consider neither the underlying etiology nor the important consequences of overeating and obesity.

Several entities have been seriously discussed with regard to the etiology of obesity, namely hypothalamic or endocrine disorders, constitutional, genetic or psychogenic disturbances or simply bad habits. Whatever the etiology of obesity, it is seldom possible to apply any causal therapy, and whenever such therapy has been attempted, it has rarely met with success. It is questionable if the eradication of an imputed hypothalamic disorder in a child has ever induced a disappearance of the state of obesity, or at least such a cure must be exceedingly rare. It has not been possible materially to influence genetic and constitutional disturbances, and hormonal treatment for obesity has not so far been crowned with success. Thus the psychogenic disturbances and family-social factors remain to be elucidated as

inductive influences in the habitual trend of overeating.

It is well known that frequently a correlation can be established between obesity in children and certain emotional disturbances. In exceptional instances they may be primary and causal, although usually they are secondary to obesity. Occasionally the emotional troubles and obesity may be unrelated to each other inasmuch as they simply constitute signs of a common constitutional aberration. It is possible that only a few obese children escape the psychic tension of an indiscreet environment's behavior toward the child. The contemporary craze for a slim figure as the ideal universal shape is bound to exert an unfavorable influence on children whose physique does not correspond with that ideal. They feel ashamed, blameworthy and shy on account of their stoutness. Fat children become easy victims of their cruel school- and play-mates' scorn, and occasionally of their parents' and brothers' and sisters' joking remarks about their ungainly body. The emotionally sensitive children react strongly to this psychic maltreatment. They consider themselves as outcasts and miserable creatures and on this account isolate themselves more and more from their comrades and their surroundings. According to Quaade

it does not appear to be the degree of fatness which determines the degree of the secondary psychic disturbance that has arisen because the child suffered from being the target of playmates' jokes, but rather the child's constitutional psychological robustness, mental assertiveness and expansive force.

Some emotionally stable children disregard and ignore their comrades' ridicule or again return tit for tat, perhaps even defend themselves with blows. Such a daring attitude is the best means of accelerating the gang's rapid acceptance of the child as it is and ignoring its fatness. Neither the stoutness nor the emotional upset appear to play any significant role in these plucky children; they have adjusted themselves and their fat body to their surroundings.

The slowness and indolence which often accompany obesity may also become an emotional and social handicap for fat children. This may further give rise to conflicts with the environment. It is primarily the teachers and the parents who find the children uninterested and dull. This leads to reprimands, reproaches, punishments, and extra lessons. In turn this increases the child's psychic tension and feelings of awkwardness and discontentment because of the isolation and maladjustment. The last vestige of activity is dissipated and the child finds satisfaction and comfort in the only enjoyment and pleasurable pastime at its disposal to brighten up its miserable existence: to eat large meals and unnecessary snacks between meals. This situation naturally fails to improve the condition.

Quite often the child's fatness is an expression of the overeating (bad) habit

of the whole family, and the end-result is that the parents as well as the children grow fat. In such usually happy and congenial families, obesity is not considered unfavorably, but on the contrary a sign of excellent health. Quaade, who in an investigation of obese schoolchildren found that only a few of these disclosed psychopathic features which might have occasioned the obesity, writes of the child's family situation as follows: "Rarely did they (the mothers) look upon the obesity as a pathological phenomenon, they preferred to regard it as a variation within the normal and of no great importance, a state of things that did not call for active measures." The parents have no thought whatsoever of reducing the weight of their fat child by means of restrictive diet, with or without the physician's cooperation. There is no nagging about eating less; there exists a harmonious concord between the members of the family and these obese children are usually quite manageable.

In other situations there is no apparent general voracity in the family, but the mother has misunderstood the child's proper food requirement and tempts it with an excessive amount of dainty and nourishing food. It is well known that one can get into the habit of eating large amounts of food at frequent meals. Aided by an imprudent mother, the child may also try to satisfy its insatiable desire for sweets. In such families the child often becomes overprotected by the mother in other respects, a situation which in itself sooner or later leads occasionally to maladjustments and the development of neuroses.

According to my personal view and experience, there is still another mechanism

ism of the development of psychic disturbances in obese children, namely, the strictly regulated dietetic regimen, a subject to which I will return a little later.

Whatever the cause, mental disorders are not infrequently encountered in obese children. Their incidence is of such a magnitude that no child should solely be treated for its obesity, without giving due consideration to the child's psychic state of health. The neglect of this dual approach is one of the reasons why treatment of obesity so often turns out to be a failure.

One may readily produce a temporary reduction in the fat child's weight by a restricted dietetic regimen, but it remains a more difficult task to maintain its normal weight curve. There are numerous reports available of a short-term successful dietetic treatment. However, there are very few long-term follow-up studies showing the importance of decreased calories and increased activity. For what reason? Mostly because of neglect to treat the whole child with its frequent emotional troubles and maladjustments and because of deficient cooperation with both the child and its family. Mothers, who are painfully aware of their son's or daughter's obesity and who long to have them become as slim as other children, often expect to achieve this end without imposing any sacrifice or inconvenience or change of daily habits on their offspring, by simply resorting to treatments with hormones or other suggested drugs. They may perhaps follow diligently over a certain period the dietetic regimen and advice recommended by the physician. But soon or later they find such counsel impossible to follow through because of the

child's resistance and emotional reaction, and eventually they give up.

It would seem to be of great importance that an attempt should be made to evaluate in advance the probable presence or absence of cooperation of both the child and the family before the dietetic restricted regimen is prescribed. If no probability exists of any long-term cooperation, I am of the opinion that one should abstain from recommending any severely restrictive diet with the aim of reducing the weight and of maintaining a normal weight. Rather the physician should confine himself to treating an eventual emotional disturbance and to advise mother and child to accept the overweight as a constitutional pattern for the individual child.

The attempt of a zealous and ambitious mother to carry through a recommended severe reducing nutritional regimen by force and punishment may call forth a feeling of revolt in the child, which may lead to serious and frequent situations of conflicts within the family. The more severe the order is that forbids something, the more intense becomes the desire to defy the order, in conformity with the adage based on ancient experience: "Forbidden fruit tastes best." As long as the child is supervised by its mother at home, in spite of protests, the child's behavior may comply with its mother's wishes. When the mother is away from home, or when the child visits in the company of comrades, the temptation to squash the order that forbids may become too strong and the child eats furtively to satisfy its hunger. Such an offence may perchance be discovered by the mother, with the result of reproaches or punishment, or that the

child feels conscience-stricken, repentant, and alarmed by being discovered by the mother, etc. Such a course of events may even make an emotionally stable child a neurotic and not diminish the corpulence as a complicated psychosomatic disease phenomenon. As long as eating amounts to an irresistible instinct and primitive gratification desire in the child, and this is not counteracted by a sufficiently great desire to restrict his overeating and hankering for sweets in order to be slim as other children, not much is accomplished by the dietetic-physical treatment of fatness in children. On the contrary the risk is great that the unwise regimen might produce maladjustment and neurosis in the child. Is it not preferable in such cases to pay more attention to the child's emotional health and harmony in the home than to its overweight?

The qualification for being able, according to the above suggestion, more or less to ignore fatness as such, is, of course, that it should not in itself imply a heavy health risk for the child. We know from adults that a fat person as a rule has a shorter life prospect than a lean person, mostly due to heart and circulation diseases. Even the increased risk at operations and the static discomfort produced by the body weight may play a certain role. Fatness favors the development of rickets in very young infants, a fact which nowadays appears to have no significance because of the generally applied D-vitamin prophylaxis. A certain infection susceptibility is characteristic of fat children. Infection susceptibility and a reduced resistance against infections, however, may be more closely associated with constitutional factors, of which fatness is a partial

phenomenon, than with fatness itself. Children's disinclination to stay out of doors at play with other children may also contribute to a lowered resistance. Thanks to the means at our disposal of warding off severe infections (sulfonamides, antibiotics, gammaglobulin), this disinclination in connection with fatness appears to play a lesser role in children than previously. Static disturbances may occur in form of flat-foot anomalies, bow-leggedness, etc. If fatness in children is not excessive, which rarely is the case, it is scarcely accompanied by any somatic troubles in contrast to the situation in adults. It is rather considered as a beauty defect. Inasmuch as one can seldom attain a permanent elimination of this uncomely appearance by a drastically restricted diet, it is far more advantageous for the child's psychic health not to attempt to alter its habits in a manner too repugnant to it. Instead one might prescribe some simple dietetic recommendations, such as, for example, decreasing the number and size of snacks, and at least restricting the use of sweets, pastry, butter and sugar at meals. If the family comprises several children, it is recommended that the prescribed dietetic regimen should be adhered to by the whole family in order to make it easier for the obese child to follow the physician's advice. The child's physical activity should also be taken into consideration in the treatment and, whenever it is feasible, it is recommended to introduce some enjoyable and well-disposed exercises. In this manner the child's family life will become harmoniously balanced.

When the child attains puberty or before it reaches sexual maturity, and its ambition is aroused and the voluntary

If a child's desire to become slimmer grows into a dominant obsession, then on its own initiative the child will cooperate in eliminating its corpulence and accept the restrictive dietary regimen with its accompanying discomforts and strains. Then it is much easier to cut down on the eating, and the temptations to backslide will not be overwhelmingly strong. So the child begins to lose weight. Even if the fatness does not always completely disappear, it becomes much easier for the child to keep the weight within reasonable bounds. In exceptional cases, fatness may during puberty even change into a pathological leanness through mental anorexia.

The correctness of the above suggestion about the treatment of obese children is proved, among other things, by the results detailed by Hilde Bruch in a follow-up study of her series of fat children. She found that 15% of the cases had become slim and showed satisfactory adaptation, 20% remained continuously fat but had adapted themselves in a satisfactory manner, 25% had become slim but disclosed emotional disturbances, and 40% remained still fat and emotionally poorly modified. She writes: "A favorable outcome, that of slenderness or moderate obesity with good social adjustment, was achieved only by those who had had the least amount of medical attention, no endocrine injections and no enforcement of dietary restriction. These patients had established control over their weight during adolescence or in early adult life on their own initiative, not under outside compulsion." The children in Hilde Bruch's series who had lost their fatness, or who under all circumstances had not been incapacitated by the same, came from homes in which

the development from infancy to maturity had not been obstructed by parental nagging, emotional tension or authoritative criticism.

All children are not alike. On the contrary, each child is an individual type. Just as slimness or overweight may occur which is considered to be of non-physiological origin, so there may also exist an overweight which separates the child from children in general. One does not know, however, whether an overweight is non-physiological just for the child in question. It may be a partial phenomenon of its bodily constitution and this cannot be altered by external means. It would be just as difficult to remove fatness permanently from an obese child by means of a relative starvation diet as it would be to make a constitutionally abnormally skinny child increase in weight and remain normal by means of an intentionally rich nutritional diet.

It is apparent from the above statements that I consider it irrational to apply immediately to obesity in childhood the suggestively most logical therapy by recommending a restricted diet. It is the obese child and not its obesity which should be treated. Our first objective is to attempt to ascertain if we are dealing with some psycho-pathological trouble in the child and to elucidate the situation between the child and the other members of its family as well as its school- and play-mates. Should there be any cause to suppose that the obesity is dependent on psychic disturbance in the child, then it is advisable to seek child-psychiatric expert help. If instead the emotional disturbance is secondary to the fat nuisance, which is usually the case, then one shall make it

known that fatness has very little significance from a physical health point of view and attempt to get the relatives and the child to accept the overweight as a constitutional anomaly which belongs to the child's peculiar make-up and try to instruct and encourage the child to ward off

his comrades' scoffing. By such measures one may hope and trust that the otherwise harmless fatness should not through unwise treatment be exchanged for an obesity neurosis or aggravate an already existing one.

### Summary

Obesity in children is often accompanied with psychic disorders and signs of family-social maladjustments. Such psychic health hazards may occasionally constitute a primary factor in the production of obesity. Usually it is secondarily produced by various psychogenic influences and mechanisms causing conflicts and tension in the family-child relationship, such as lack of sufficient understanding, ill-treatment of the child's corporal handicap or rash attempts to reduce the weight without the joint effort of the child. In view of the fact that obesity rarely constitutes a serious physical health hazard for the child, it would seem more appropriate to omit any strictly regulated reducing diet. Treatment should primarily concern the obese child and not solely its obesity.

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## Myocardial Changes Following Oxygen Inhalation

### An Experimental Study on Rabbits

by R. BERFENSTAM and L. ZETTERGREN

Liebegott (1940) studied those organic changes which arose after exposure to an atmosphere containing about 85 per cent of oxygen at N.T.P. for 7 days in various species (albino mouse and rat, guinea pig, rabbit, cat and dog). At histological examination of the rabbit hearts he found severe changes in the form of necrotic foci localized predominantly in the apical portion of the left ventricle including the papillary muscles. The necrotized muscular tissue was replaced by young fibroblasts, polymorphonuclear leukocytes and round cells. Liebegott ascribed the observed myocardial changes—which were described rather summarily—to hypoxia due to pulmonary lesions induced by inhalation of excessive oxygen concentrations.

In the same year Pichotka (1940) reported the results of a similar investigation where guinea pigs exclusively had been used. No myocardial changes could be demonstrated, however. Pichotka presumed that the absence of such changes might have been due to the fact that the hypoxia resulting from exposure to oxygen had been too brief.

Considering that no penetrating studies

of cardiac changes following exposure to excessive oxygen concentrations seem to have been published and because spontaneous myocarditis (pen infection) is a possibility that had not been ruled out in Liebegott's investigation, we have felt it justified to report the results of such a study. In a forthcoming publication it is hoped to supplement these findings with electron-microscopic observations of the early changes induced by oxygen inhalation.

### Materials and Methods

Forty-six adult, mixed-breed rabbits of both sexes, whose body weights varied from 2.0 to 3.5 kg and averaged 2.5 kg, were used for the experiments. The animals were given food and water *ad libitum*.

Exposure to oxygen was accomplished in a gas chamber of 0.3 m<sup>3</sup> capacity provided with a closely fitting glass cover. In all the experiments a temperature of 20° to 23°C and relative humidity of 70 to 80 per cent were maintained within the chamber. Oxygen at atmospheric pressure from a battery of gas cylinders was supplied at a rate enabling its concentration in the chamber atmosphere to be kept between 70 and 80 per cent. Separate inlet and outlet ports on the chamber were interconnected by a closed

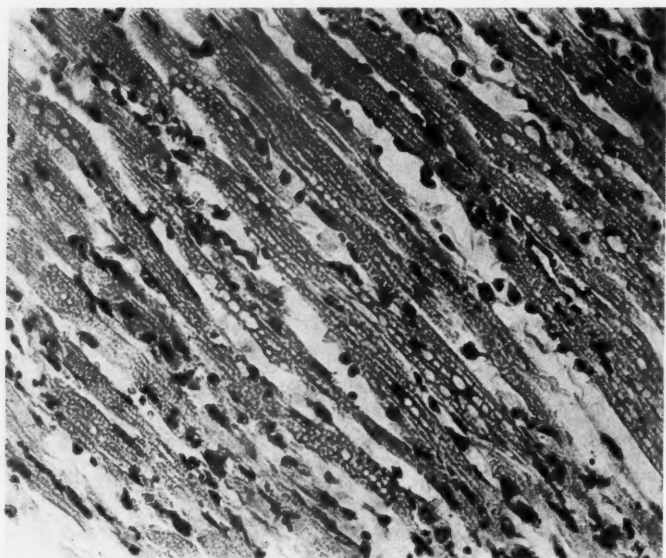


Fig. 1. Myocardium with vacuolar degeneration. Hematoxylin-van Gieson.

circulation circuit comprising a pump, water trap and carbon dioxide absorber. The pump was driven by a motor incapable of generating ozone.

As soon as possible after death the animals were dissected and heart and lungs excised. After fixation in 10 per cent formalin solution and/or absolute ethanol, parts of these organs were embedded in paraffin, sectioned and stained with hematoxylin-van Gieson's stain, Heidenhain's iron-hematoxylin, Best's carmine and periodic acid-Schiff (PAS). Other parts were frozen, sectioned and stained with scarlet red.

### Results

When the rabbits had been exposed to the high- $O_2$  atmosphere in the gas chamber for about 48 hours, they began to refuse food and water, their respiratory movements became slow and deep, they crouched quietly, and sometimes their heads were maximally dorsiflexed. These signs

became more pronounced with time, and after the rabbits had been in the gas chamber for 72 to 144 hours death occurred.

If rabbits in this state were removed from the gas chamber, their respiratory distress would increase and they occasionally expired in a condition resembling pulmonary edema.

### *Post mortem observations*

*Gross findings.*—The pleurae contained small amounts of a colourless liquid. The lungs were enlarged, of bluish-red hue and had a fairly firm, hepatoid consistency. Comparatively large amounts of a foamy, colourless liquid were present in some animals' trachea and bronchi.

Most of the hearts were enlarged, chiefly because they were dilated and heavily congested. The pericardia usually contained

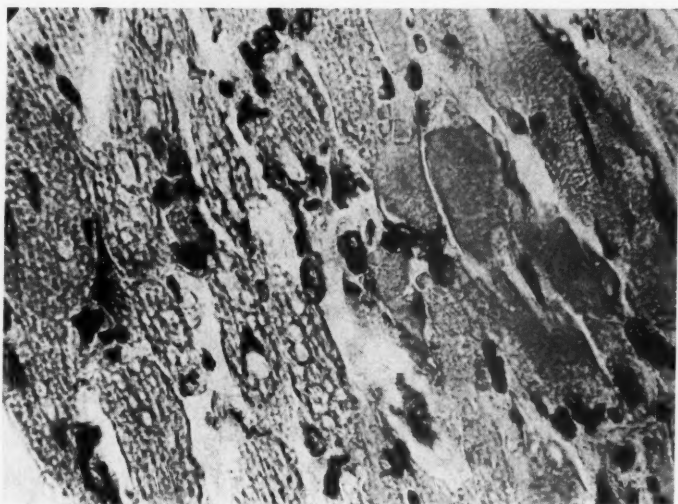


Fig. 2. Myocardium with vacuolar degeneration and necrosis of occasional muscle fibres. Hematoxylin-van Gieson.

some colourless liquid, but their surfaces were smooth and glistening.

*Microscopic changes.* — Microscopic myocardial changes were noted in 38 of the 46 experimental rabbits. These were severe in 8 animals (Group I), moderate in 12 (Group II) and slight in the remaining 18 (Group III). Although they occurred in all parts of the heart, the lesions were consistently most pronounced in the left ventricle, especially its apical portion.

The most frequent type of lesion had the appearance of *vacuolar degeneration* (Fig. 1). Such degeneration was encountered in all the rabbits of Groups I and II and in some of those of Group III. Just as in lesions of other types, the vacuolar degeneration was focal rather than generalized, but lacked manifest topographical relations to vessels, endocardium or pericardium. In the earliest stages of vacuolar degeneration, minute vacuoli which were

uncoloured and transparent as water, often disposed in rows like a string of pearls, were observed between the muscular fibrils. Whereas the striation of muscle fibres which were thus changed was on the whole preserved, their thickness was somewhat greater than normal. In rabbits with more advanced lesions, the vacuoli were larger, some of them twice the size of an erythrocyte. As a rule the largest vacuoli were situated next to the nuclei and sometimes formed a depression therein (Fig. 1). Muscle fibres exhibiting this advanced form of vacuolar degeneration were considerably thickened and their striation only partially preserved (Fig. 2). The vacuoli mentioned in the foregoing contained neither glucogen nor lipids.

Areas with pronounced vacuolar degeneration in some of the animals were found to be homogenized and completely devoid of structure (Fig. 2). In addition such areas

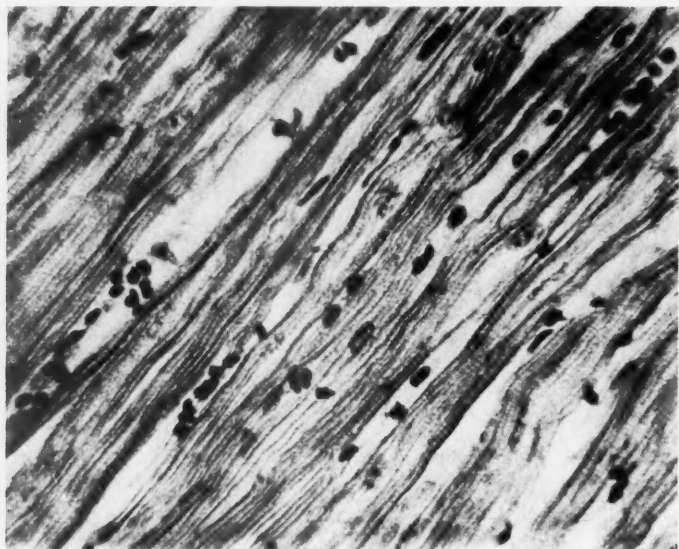


Fig. 3 Muscle fibres with thickened fibrils. Hematoxylin-van Gieson.

often presented varying degrees of *interstitial edema* that had torn apart the muscle fibres and connective tissue fibrils.

Another frequent finding was *altered stainability* of the muscular tissue, affecting either occasional fibres or small circumscribed areas which sometimes were situated perivascularly. The altered stainability was manifested by enhanced affinity for eosin and also for PAS causing the muscular tissue to take on a bright pink colour. Although the majority of areas whose stainability was altered in this way exhibited no structural changes, some of them were observed to show early vacuolar degeneration. Attempts to stain such areas for glycogen and lipids were unsuccessful.

Other and usually very varying forms of *major structural changes* were encountered in all the rabbits in Group I and in a

few of those in Group II. Thus, in certain areas, the striation of the muscle fibres had been obliterated, their thickness varied within wide limits, and the fibrils were thickened (Fig. 3) and abnormally stained by iron-hematoxylin (Fig. 4). Elsewhere in the myocardium the fibres exhibited marked granulation or fine reticulation (Fig. 5). Fractionated iron-hematoxylin staining revealed that in areas of the latter appearance the normal structure of the muscle fibres was completely destroyed, with the sarcoplasm undergoing scaling and mossy disintegration (Fig. 6). Ultimately this apparently rather slow myolysis with the concurrent interstitial connective tissue cell proliferation, histiocyte mobilization and lymphocyte and plasmacyte infiltration gave rise to lesions resembling those seen in myocarditis (Fig. 7). Being most pronounced in the apical



Fig. 4. Muscle fibres with thickened fibrils and varying stainability.  
Heidenhain's iron-hematoxylin.

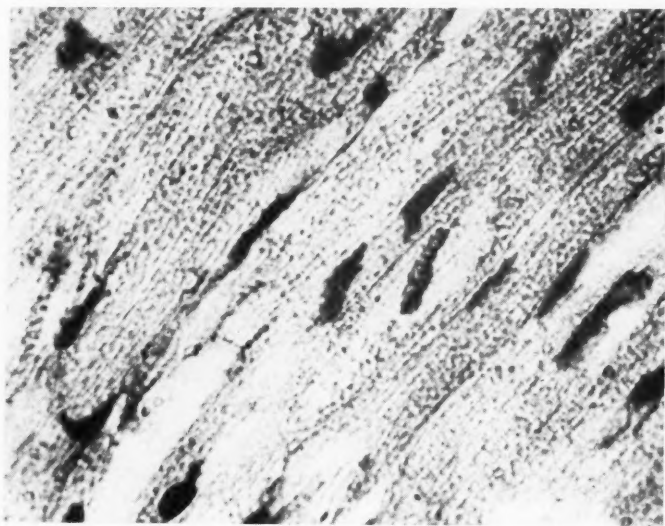


Fig. 5. Muscle fibres with fine reticulation. Hematoxylin-van Gieson.



Fig. 6. Muscle fibres with scaling and granular disintegration.  
Heidenhain's iron-hematoxylin.



Fig. 7. Myocardium exhibiting necrotic foci with infiltration of inflammatory cells.  
Hematoxylin-van Gieson.

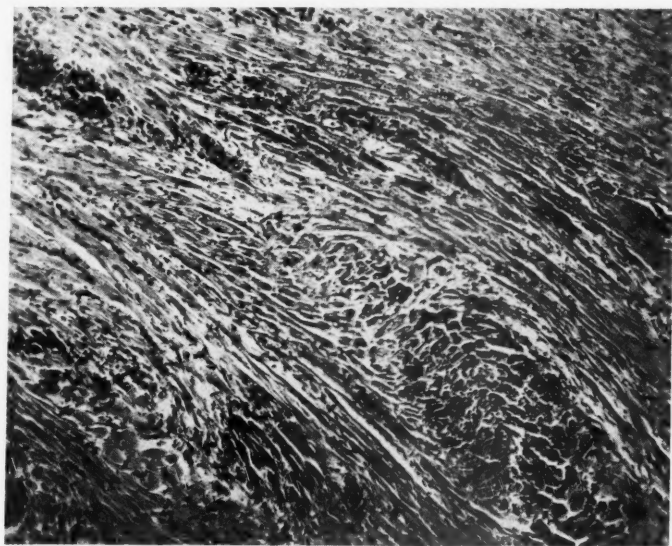


Fig. 8. Myocardium with necrotic foci with calcifications.  
Hematoxylin-van Gieson.

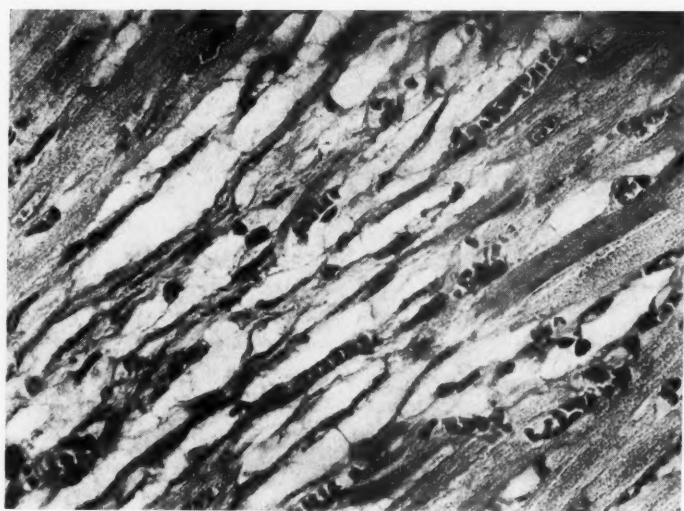


Fig. 9. Myocardium with myolysis, but no inflammatory reaction.  
Hematoxylin-van Gieson.

portion of the left ventricle including the papillary muscles, such myocarditoid foci were often situated perivascularly. In some of the rabbits in Group I, furthermore, small, circumscribed areas of necrosis without reactive changes were observed. Calcifications were seen in some of these necrotic foci (Fig. 8). In some animals, finally, small areas were encountered in which the muscle fibres had been completely or almost completely destroyed and yet the mesenchyma remained intact (Fig. 9).

When stained for lipids, sections of myocardium from all groups, but particularly often those from Group I, were found to include small areas of fatty degeneration.

Glucogen staining revealed that the muscular tissue bordering on areas of myocarditic appearance was often very rich in glucogen. Apart from this, however, no manifest deviations in glucogen content were noted.

#### Controls

For the purposes of another investigation, a large number of identically raised rabbits of the same stock were killed. It was convenient to use 9 of these rabbits as controls in the aforementioned experiments. It appeared that none of them exhibited myocardial changes of any of the types described here.

#### Discussion

From the trials described in this paper it has appeared that in the rabbit prolonged exposure to excessive oxygen concentrations gives rise to myocardial changes of very varying character. An important factor in the genesis of these changes is the increasing respiratory distress seen in animals that have been in-

haling oxygen at an elevated partial pressure for two or three days. This condition might be due to oxygen-induced pulmonary lesions, for example atelectasis and hyaline membranes (Liebegott, 1940; Pichotka, 1940; Berfenstam *et al.*, 1954, 1958; De & Anderson, 1954), resulting in impaired gas exchange in the lungs. Hence the ultimate outcome of oxygen inhalation would be so-called paradoxical hypoxia. Moreover, it has been shown experimentally that the arterial oxygen tension in dogs is reduced to 38 per cent after 8 days' inhalation of 80 per cent oxygen (Binger, Faulkner & Moore, 1927). In the present series of experimental animals, the lungs were also examined microscopically, the degree of atelectasis and the incidence of hyaline membranes being noted and correlated with the severity of myocardial changes. Without going into the details of this analysis, we would emphasize that the extent of pulmonary lesions showed a definite though not total correlation with the severity of myocardial changes. Owing to such things as lack of sufficient physiological data, our ability to establish the temporal relations of the development of these pulmonary and cardiac changes remains very limited. Nevertheless, we feel justified in presuming for the time being that the myocardial changes are secondary to the oxygen-induced pulmonary lesions and accordingly caused, at least to some extent, by hypoxia. This assumption is borne out by the prevalence of vacuolar degeneration in the myocardium of the experimental animals for it has been demonstrated that hypoxia causes vacuolar degeneration (Büchner, 1932, 1933; Hansenberg, 1939; Kritzer, 1944; Lewis & Haymaker, 1948; Grundmann, 1949). The

other observed myocardial changes, too, that they at least partially could be due must probably be attributed to hypoxia, to metabolic disturbances following the even though it cannot yet be ruled out oxygen intoxication.

### Summary

A variety of myocardial changes, predominantly in the left ventricle, were observed in 38 of 46 rabbits which were exposed to 70 to 80 per cent oxygen until they died. In the majority of animals the changes took the form of vacuolar degeneration, altered stainability of the muscular tissue and interstitial edema. In addition, the animals exhibited major structural changes leading to myolysis and development of myocarditis-like foci. It is the authors' contention that the changes in question are secondary to such pulmonary lesions as are caused by oxygen inhalation and, hence, that at least partially they are caused by hypoxia.

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## Atypical Patent Ductus Arteriosus in Infancy

by ÅKE GYLLENSWÄRD

The term *atypical patent ductus* has become the generally accepted mode of describing cases of patent ductus arteriosus with pulmonary hypertension but no other anomalies of the heart or great vessels. Since patent ductus was the first cardiac malformation to become treatable by surgery, it is natural that its variations have received considerable attention. Nonetheless, a long time elapsed before the serious clinical pictures that can appear even during infancy, and that may be due to grossly patent ductus alone, were recognized. In a few publications, however, it was early pointed out that patent ductus is not uncommonly the sole cause of death (Abbott, 1937; Wilson & Lubschez, 1943). Such cases are often misunderstood by inexperienced examiners, who interpret the secondary and terminal pulmonary changes as the main cause of death.

Since the beginning of the 1950's, the clinical picture has been repeatedly described in the cardiological literature (Ziegler, 1952; Dammann & Sell, 1952; and others), and is to be found in up-to-date handbooks on paediatric cardiology (Kjellberg, Mannheimer, Rudhe & Jonson, 1955; Nadas, 1957; Keith, Rowe & Vlad, 1958). It would seem, however, owing to specialization in medicine and medical literature,

that this knowledge has spread little outside cardiological circles. Despite the tremendous advances in cardiac surgery, practically the only cases of congenital cardiac anomaly with poor short-term prognosis among infants, that can at present be successfully treated by operation are those of atypical ductus arteriosus. It is therefore of the greatest concern that these particular cases are singled out for specialist treatment. In order that the diagnosis be made and operation carried out at an early stage, it is essential that the condition be recognized by the paediatrician.

## Material

The series to be described consisted originally of 14 cases aged from 3 to 17 months, diagnosed as atypical patent ductus arteriosus with no other cardiac anomaly. Of these, 3 were boys and 11 girls, which represents the normal sex distribution in patent ductus. It is noteworthy that 3 of the 14 mothers had had rubella during the first or second month of pregnancy. All of their children had, in addition to the cardiac lesion, unilateral or bilateral cataract and microphthalmos, and were generally underdeveloped. Another child was deaf, and yet another mentally retarded. One of the mothers had severe diabetes mellitus, and had several times been in coma during the early part of the pregnancy. In her child a large ventricular septal

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defect that had been overlooked even at cardiac catheterization was subsequently found, and this case was therefore excluded from the series (Case 14). Of the remaining 13 cases, not fewer than 5 had severe congenital malformations of organs other than the heart.

In most of the cases the birth-weight was strikingly low, even though none of the infants was born more than two weeks before the expected date. The mean birth-weight for the series was 2700 g (2100-3900 g).

It is of particular interest that 6 of the 13 children belonged to the region served by the Uppsala clinic, the remaining 7 having been referred from other parts of the country where there is no specialist in paediatric cardiology. The ratio between the populations of the Uppsala district and the other districts is 2:15. Since no further infants with atypical patent ductus were referred to other paediatric-cardiological centres from these districts during the period covered by the investigation, there is reason to presume that the condition is going unrecognized and infants are dying of it. This contention is borne out by the fact that older children with atypical patent ductus are now rarely seen in Sweden.

Our cases are presented in Table 1.

### Clinical Picture

Briefly, the clinical picture is as follows. The infant is very poorly, and from the start is difficult to feed and shows little weight-gain. Attacks of cyanosis are common during the neonatal period, but these disappear, and do not recur until the pressure in the pulmonary artery equals that in the aorta, and this does not usually take place during infancy. Recidivating, protracted pulmonary and bronchial infections, and especially asthmatic bronchitis, are common, and the child may become very ill. Even during uninfected periods mucous secretion is increased in the bronchi. Precordial bulging and thrill

are common, but the latter is often difficult to detect owing to the prominent bronchial signs and the inevitable tachypnoea. For the same reasons, auscultation of the heart is as a rule difficult. The continuous murmur typical of patent ductus arteriosus is often absent. Instead there is a loud, rough, prolonged systolic murmur, which is loudest over the fourth left interspace, and which is characteristically heard more plainly over the base of the heart than over the apex (Fig. 1). In addition it may continue into the second sound, but this can rarely be established without a phonocardiogram. A mid-diastolic murmur is also often heard in the region of the apex (Ravin & Darley, 1950; and others). The less pronounced the pulmonary hypertension is, the more continuous does the murmur become. Even in cases with marked pulmonary hypertension the phonocardiogram may indicate a continuous murmur over the 2nd-4th left interspaces, although it is heard as a pure systolic one. Most writers claim that the murmur is most commonly purely systolic, but their views are usually based on auscultatory findings alone. In 7 of our 13 cases phonocardiography revealed an indisputable continuous murmur, and in 3 more the systolic murmur tended to persist over the second sound. Even in cases of typical patent ductus, the murmur during infancy often seems to be purely systolic, changing to a continuous murmur after the first year of life. Phonocardiography, however, often reveals that the apparently systolic murmur is in fact a continuous one, with a very short diastolic component.

The electrocardiogram reveals hypertrophy of both right and left ventricles

TABLE 1.

Key to abbreviations: C = continuous murmur, S = systolic murmur, D = ductus divided, L = double ligature and transfixation.

No.	Age, mths.	Sex	Birth weight g	Clinical data				X-ray	ECG		Catheter pressures, <sup>a</sup> mm Hg			Surgery	Postop. course	Remarks
				Growth impairment	Respiratory infection	Type of murmur at area of max. intens	Apical, mid-diast. murmur		Heart volume cc/sq.m surface area	Left ventr. hypertr.	Right ventr. hypertr.	RV	PA			
1 253/54	4	F	3900	+	+	S	—	350	+	(+)	(17)	(39)	(71)	L	Rapid improvement and gain in weight. Postop. X-ray and ECG normal	Asthmatic bronchitis
2 387/56	17	F	3300	+	+	C <sup>b</sup>	—	390	+	—	24/0 (9)	26/19 (20)	75/56 (65)	L	"	"
3 546/56	16	F	2700	+	—	C	—	350	+	(+)	No cathet.			L	"	Mentally retarded
4 1012/56	9	M	2500	++	+	S	(+)	515	+	+	70/0 (20)	70/35 (45)		D	Died 2 hrs postop.	Necropsy revealed no further anomaly
5 464/57	4	F	2900	+	—	S	+	500	+	+	No cathet.			No op.		Left cataract. Mother rubella mens II. Died at 6 mths. Diagn. continued
6 783/57	5	F	2100	—	+	C	—	230	+	(+)	49/0 (20)	43/7 (40)	88/60 (74)	L	"	"

Case	Age	Sex	Weight	Temp.	Pulse	B.P.	Heart	Respir.	Diagn.	Remarks
783/57	7	F	2100	-	+	+	C	+	L	"
1272/57	8	M	3150	-	+	+	C <sup>c</sup>	+	D	Motheritis mens II-III
1303/57	9	F	3100	+	+	+	C	+	D	Deaf
1311/57	10	F	2200	+	+	+	S	+	No op.	Died at 9 mths. after angiocardio-graphy. Diagn. confirmed at necropsy
1443/57	11	F	2400	++	+	+	S	+	D <sup>d</sup>	As in case 1
1502/57	12	M	2300	++	+	+	S	-	L	Cataract bilat. Mo-ther rubella mens I.
669/58	13	F	2150	++	+	+	C	-	D	"
	14	F	3100	++	+	+	S	+	D	Slight pul-monary stenosis
	15	F	3100	++	+	+	S	+	D	Postop. ca-thet. show-ed ventr. sept. defect

<sup>a</sup> Mean pressures within parentheses.

<sup>b</sup> S 6 months previously.

<sup>c</sup> S 3 months previously.

<sup>d</sup> Cardiac arrest at the beginning of the operation.

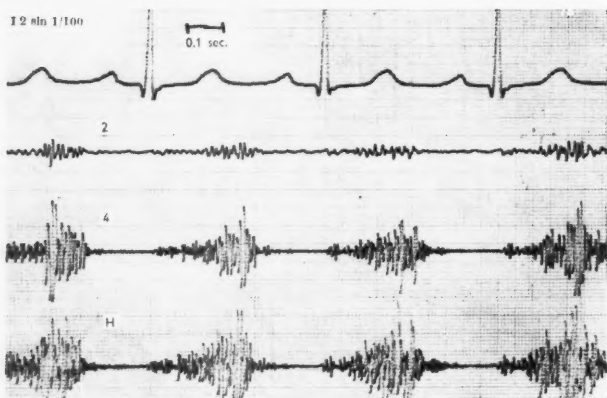


Fig. 1 A.

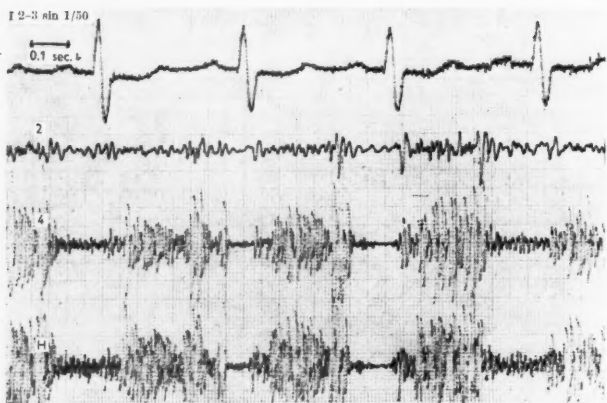


Fig. 1 B.

Fig. 1. (A) Continuous murmur with a short diastolic component (Case 6). (B) Systolic and diastolic murmurs. (The diastolic murmur was more pronounced over the apical region.) (Case 11.)

(Fig. 2). The direction of the electrical axis may vary greatly.

The heart is considerably enlarged and the volume may become immense. In one of our cases it was 650 ml/sq.m body surface. The enlargement involves the left ventricle and left atrium in particular, but the right ventricle is also enlarged. The pulmonary artery is widely dilated, and

the vascular shadowing in the lungs increased (Fig. 3). At the same time there are not uncommonly opacities in the lung fields due to patches of broncho-pneumonia and atelectasis.

#### Discussion

Simple patent ductus arteriosus can with certainty be diagnosed with the aid of

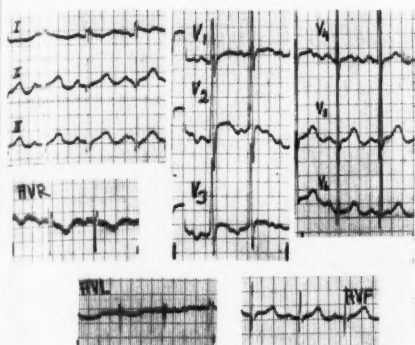


Fig. 2. A usual ECG in atypical patent ductus arteriosus during infancy (Case 9).

the stethoscope alone. Atypical patent ductus, however, requires further investigation, partly because it may easily be mistaken for a large ventricular septal defect, and partly to exclude the presence of other anomalies, especially ventricular septal defect (Dammann & Sell, 1952; Ziegler, 1952, Young, Rowe, Curreri & Gale, 1958) and pulmonary stenosis. Cardiac catheterization, especially in young infants, may present great difficulties of both execution and interpretation. The pulmonary hypertension is, of course, always accompanied by raised pressure in the right ventricle. Conditions of flow at the tip of the catheter may cause a reading in the pulmonary artery 10–15 mm Hg lower than under ordinary circumstances, even in the absence of pulmonary stenosis (Jonson, 1957). And, on the other hand, even quite severe pulmonary stenosis may be associated with a quite moderate pressure gradient between the right ventricle and pulmonary artery, since the pressure in the pulmonary artery is influenced by the widely patent ductus. In such a case, certainty can only be reached with the

aid of selective angiocardiology from the right ventricle. Increased oxygen saturation in the pulmonary artery may be due to a very high ventricular septal defect, even if shunting to the right ventricle cannot with certainty be demonstrated. In some cases, in which there is gross shunting to the pulmonary artery, insufficiency of the pulmonary valves arises. The saturation values in the right ventricle will then be similar to those obtained in cases of high ventricular septal defect. It is often possible to pass the catheter through the ductus, thus demonstrating its presence. In order to demonstrate or exclude co-incident ventricular septal defect, it is necessary to perform selective angiocardiology with very rapid injection of the contrast medium into the out-flow tract of the right ventricle, and with rapid sequence of exposures. Even this technique may miscarry when the shunt is from left to right under physiological conditions. The smaller the difference in pressure between the ventricles, the greater the chances of demonstrating the defect. In cases where the systolic pressure is the same or nearly the same in the pulmonary artery and aorta, no shunt can be demonstrated by catheterization, but selective angiocardiology usually gives the diagnosis. Venous angiocardiology is inadequate, owing to the haemodynamic conditions described above. Pulmonary hypertension of primary nature or secondary to pulmonary disease can often not be excluded pre-operatively, but those patients are usually cyanotic from an early stage. In such cases, as when a large ventricular septal defect maintains the pressure in the pulmonary artery, the pressure-fall in the pulmonary artery that can

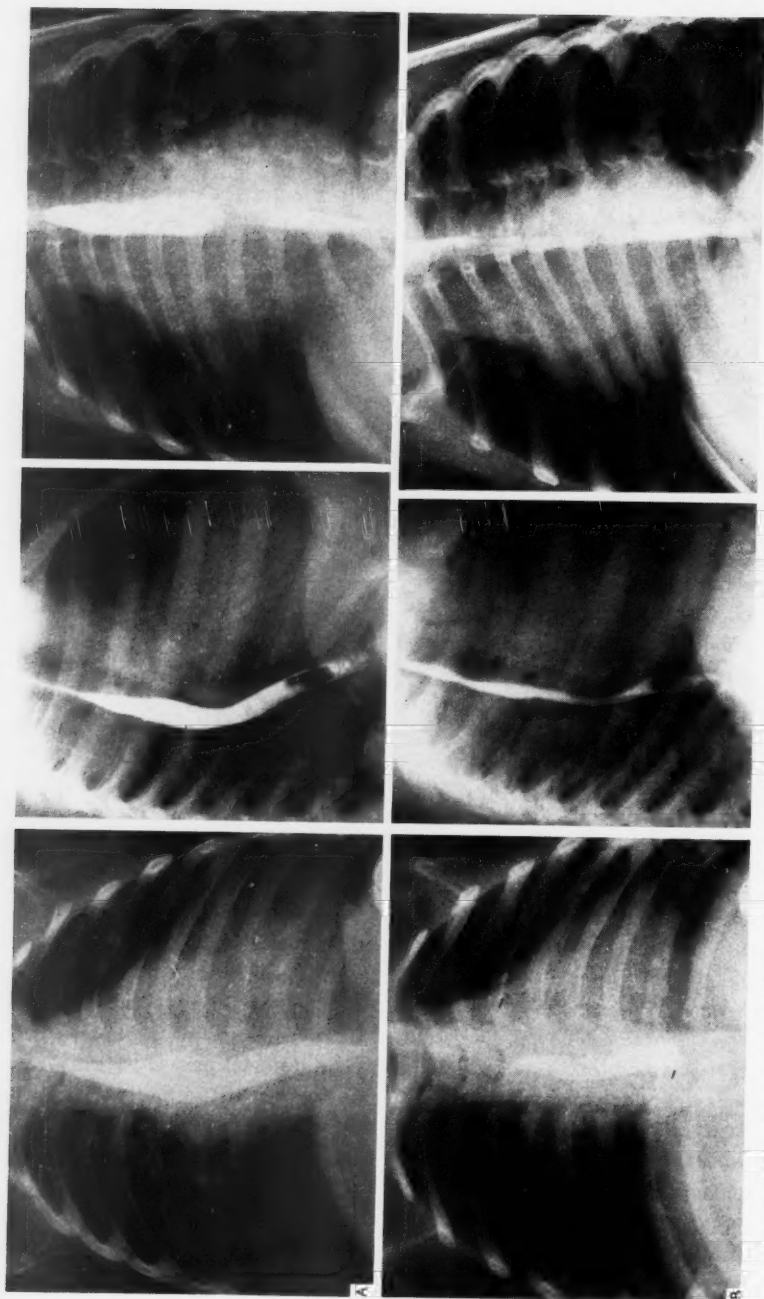


Fig. 3. X-ray of heart. (A) before and (B) 6 months after operation. Relative cardiac volume 400 and 200 cc respectively (Case 13).

otherwise be recorded when the ductus is clamped does not take place.

The question of the aetiology of the pulmonary hypertension in cases of atypical patent ductus arteriosus will not be entered into here. It may be pointed out, however, that the phenomenon may have different causes in different cases. There may be a primary lesion of the arterioli of the lungs, causing the fetal vessel walls to persist; or the hypertension may be an effect of the excessive flow, which causes both spastic and later on organic changes, with increased resistance as result (Civin & Edwards, 1951). The chances of the pressure in the pulmonary artery becoming normal after closure of the ductus are greater in the case of the latter alternative, especially if the organic changes have only progressed slightly. It is probable that the former alternative leads to a right-to-left shunt during the first years of life. In patients with right-to-left shunting, regression of the pulmonary hypertension may not be expected after closure of the ductus, and are therefore not suitable for operation. Further, there is no regular correlation between the calibre of the ductus and the degree of pulmonary hypertension. Patients are thus encountered in whom the ductus is short and widely patent, but who have only slight pulmonary hypertension at one year of age, whereas there are others with high pressure in the pulmonary artery and a much narrower ductus.

Since operative treatment of infants with congenital heart disease has not been very encouraging, even with up-to-date methods, clinicians at many cardiological centres take a negative attitude to cardiac catheterization and angiocardiography

during the first year of life. The techniques are difficult, and the results not easily evaluated; and the risks are not to be disregarded. The possibility of atypical patent ductus arteriosus with no accompanying anomalies is easily overlooked. Among our cases there are two that were judged to have a complex cardiac malformation. They were in poor condition, and detailed investigation was not undertaken. These infants died at 6 and 9 months respectively (Cases 5 and 10), and necropsy revealed that nothing more than very widely patent ductus arteriosus had been responsible for the grave illness. Subsequently, in both cases, it was found that the clinical signs, and electrocardiographic and roentgenological findings fit in very well with the clinical picture of a gross atypical patent ductus arteriosus. In one of the cases we might defend ourselves with the excuse that the parents of the infant were not in favour of cardiac catheterization, but this would be the cry of the unrepentant.

The results of operation in our series are similar to those reported by others. Of the 11 infants treated by surgery, one died two hours after operation, and the remaining 10 are perfectly healthy or greatly improved, that is to say, the symptoms and signs ascribable to the cardiac anomaly have disappeared. It is true that only one case in the final series has been recatheterized, but the clinical picture together with the electrocardiogram and roentgenograms are enough to assess the result. Immediate clinical improvement follows operation, but several months may elapse before the X-ray and electrocardiographic findings become normal. In most cases there is a residual soft systolic mur-

mur over the 3rd left interspace. In only one case was there evidence that this was due to pulmonary stenosis (Case 13); in the others the murmur was with all probability caused by a dilated pulmonary artery (Ekström, 1952). The infant with the concomitant ventricular septal defect stood the operation remarkably well, and is still alive one year later. Follow-up examination 4 months after operation even showed some improvement (Case 14). The infant that died, a boy of 9 months (Case 4), was one of our first patients, and had a greatly enlarged heart and signs of gross decompensation. He died of pulmonary oedema, and it is not unlikely that he would have survived had we followed the present exceedingly stringent rules concerning administration of fluid during and after operation. At that time we did not realize how very restrictive one must be in this connexion. The reluctance to operate upon cases of atypical patent ductus which is still encountered

in some centres would therefore seem to be unjustified to-day. The mortality of the procedure is less than 10 %, and untreated the prognosis is very much worse.

In every case, therefore, where there is the least suspicion of atypical patent ductus arteriosus detailed cardiological investigations should be carried out. The time for this depends upon the infant's condition, and will therefore often be before 6 months of age. It should be pointed out, however, that children with patent ductus with a gross shunt may, like children with ventricular septal defect, be greatly incapacitated during infancy, but may develop fairly normally during the following period, the increasing pulmonary hypertension not becoming evident until several years later. Patent ductus arteriosus, at any rate when diagnosed during childhood, should always be treated by surgery, but it may be extremely difficult to choose the right time. In uncomplicated cases, the preferred age is 3-7 years.

### Summary

A brief account is given of atypical patent ductus arteriosus in infancy. It is presumed that there must be many cases that are never diagnosed or treated, and that are not recognized even post mortem.

Thorough cardiological investigation should be undertaken in all acyanotic infants where there is the least suspicion of atypical patent ductus arteriosus. With the aid of phonocardiography it can be demonstrated that a continuous murmur is commoner among infants with atypical patent ductus than was formerly believed. The operative risks are probably very much smaller than is generally supposed.

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## Electrocardiographic Studies in the Healthy Newborn

by MAGNUS MICHAËLSSON

A considerable number of investigations have been published concerning electrocardiography in the healthy newborn. We believe the outstanding contribution in this field to be that of Ziegler (1951). Differences in the performance and results of the present investigation seem to justify the presentation of yet another study on healthy children. A review of the literature and a comprehensive list of references can be found in Ziegler's book. For works which appeared after 1951, see Rossi (1954), Knipping *et al.* (1955) and Nadas (1957).

## Material and Methods

The apparatus used was a direct-writing, two-channel electrocardiograph with jet recorders (Elmqvist's Mingograph Model 23, manufactured by the Elema Co., Stockholm). The paper speed was in every case both 50 and 100 mm. per second and the accuracy of the speed was tested a couple of times during the experiments. The precordial leads were recorded with circular silver electrodes. In order to avoid surface conduction, the diameter of these electrodes was only 8 mm. They were fastened with a piece of plaster of about 2 sq. cm. Care was taken to avoid excessive amounts of electrode paste, and the surface of the precordium was cleaned between each set of recordings. The following 12 leads were used at each recording period: I, II, III, aVR, aVL, aVF, V1, V2, V3, V4,

V5, V6. The standardization 1 mV = 1 cm was controlled repeatedly for each lead.

The experiments were carried out on 56 babies (26 males and 30 females) born at the University Hospital in Uppsala during the period December 1957 to January 1958. This figure represents about 25% of all children born in the hospital during that time. Only full-term babies uneventfully delivered of healthy mothers have been included in this study. All electrocardiographic recordings were made by the author personally. When an opportunity was given to make recordings, these were made on the two or three youngest babies born on that day. The distribution of the children according to birth weight is given in Fig. 1. The physical findings were normal in all cases. Unavoidably the experiments were irritating to the child, especially during recording of the precordial leads. With two exceptions the child could be calmed, at least for a short while. As a rule, the condition of the child changed

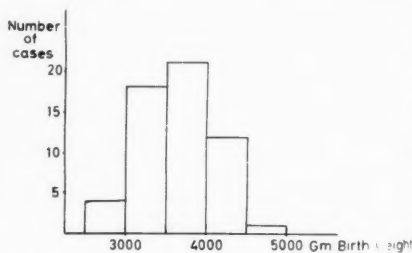


Fig. 1. Distribution of the material according to birth weight.

during a recording period between more or less shallow sleep and vigorous crying. In most cases the recording was made within two hours after a meal. All recordings were made with the subject in a supine position, and no sedative or other medication was used in any case. The investigation time was 30-60 minutes on each occasion. The recording time was between 4½ and 10 minutes. The first electrocardiogram was recorded within 30 minutes after delivery in 8 cases, within 3 hours in 40 cases, and in the rest within 16 hours. The subsequent electrocardiograms were recorded on the second, third, fifth and seventh days. In 37 cases an electrocardiogram was recorded 5 times on each child and the number of electrocardiograms per child was never less than 3. The total number of electrocardiograms was 252.

### Results

The electrocardiographic data have been correlated with medication to the mother, duration of labour, birth weight, loss of weight and time from last meal. The only correlation to be found was a slightly lower heart rate after the meal. All data have been compared with regard to the day of life and this comparison will be presented under each heading separately.

#### *Rate and rhythm*

The state of the child with frequent alternation between sleep and crying at each recording was reflected in the heart rate, which was measured in periods of six seconds all through the recording time. As a rule, the rate was high in precordial leads (the child being irritated by the manipulation). Fig. 2 is a fairly representative example of heart-rate fluctuations during the recording time. Fig. 3 shows the difference between minimum and maximum heart rates at various ages. In the group

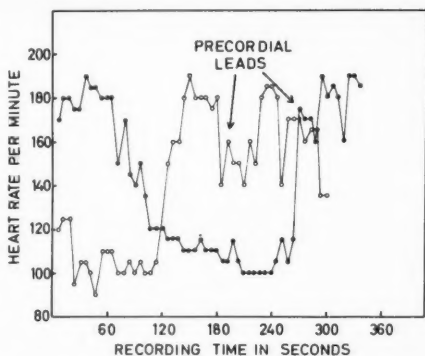


Fig. 2. Heart-rate fluctuations all through the recording time on the first (filled circle) and second (open circle) day in the same baby.

as a whole there was a slight tendency to more stable rates in the first day with an increase of the differences in the following days. This difference kept fairly constant from the second to the seventh day, considering the group as a whole, but there

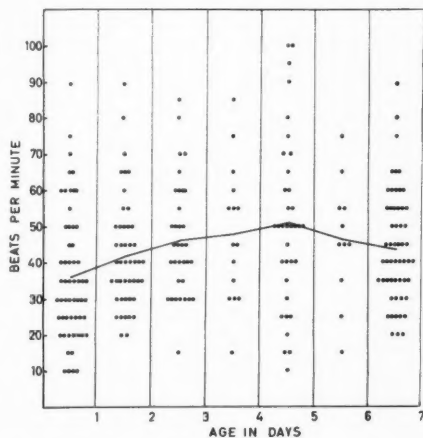


Fig. 3. Difference between minimum and maximum heart rates on various days of all recordings. The line connects the mean values of the single days. The mean value of the first day becomes even lower (from 36 to 25) if one takes into account the measurements performed within 30 minutes after birth.

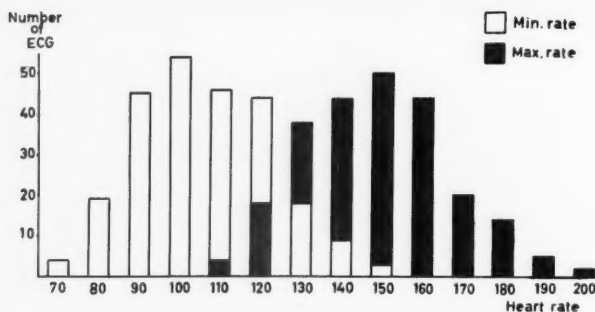


Fig. 4. Minimum and maximum heart rates of all recordings.

were large variations in the individuals, quite regardless of age. There was almost no difference between minimum and maximum heart rates when the various days were compared. The complete results (all the 252 recordings) are presented in Fig. 4 where minimum and maximum rates measured in periods of six seconds are reported. The rates were found to be respectively lower and higher than in similar reports by other authors. The essential reason seems to be our longer recording time. When the rate was measured only between two QRS complexes instead of in periods of six seconds, the minimum rate was at least 10–20 beats lower and the maximum 10–20 higher, except at the extreme values.

Sixteen cases showed more or less regularly recurring sinus arrhythmia, with differences of heart rates between 20 and 45 beats per minute. This arrhythmia did not occur on any special day and did not always occur on all days in the same child. As could be expected, the sinus arrhythmia was more common in low heart rates. The arrhythmia was independent of the respiration, as Nordenfelt (1943) has shown to be the case with sinus arrhythmia in

children less than 1 year old. It is interesting to note that Nadas says that sinus arrhythmia is almost never seen in infants. The high frequency of sinus arrhythmia in these cases seems to depend on the long recording periods and the lower heart rates.

Isolated sinus arrest was noticed in one case and a few premature supraventricular systoles in three cases. Ventricular ectopic beats occurred in two cases, one of them with just a few beats. The other case proved to be of special interest. From the first electrocardiogram at two hours of age, there appeared an accumulating number of escaped beats. On the seventh day there were 200 ectopic beats in 6 minutes (25% of all beats) and as shown in Fig. 5 the type was ventricular fusion beats partly occurring as bursts in bigemini position. At 14 days of age, the electrocardiogram was quite normalized. Nothing was observed on physical examination in this child, not even during the occurrence of ectopic beats.

In 12 cases supraventricular arrhythmias were registered, in which the P wave changed greatly in height, and sometimes the duration of P and P–R interval varied

Fig. 5

Fig.

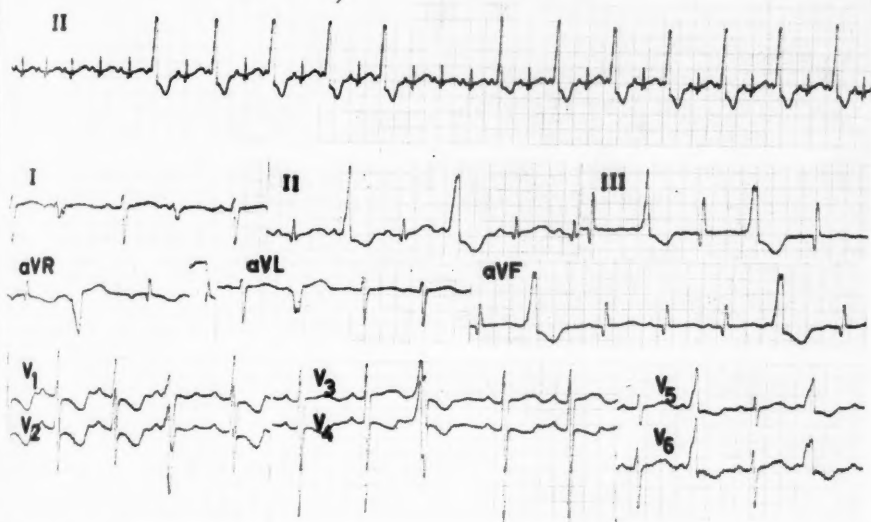


Fig. 5. The electrocardiogram on the seventh day of the baby with ventricular fusion beats. Paper speed: upper lead II, 25; the other leads, 50 mm per second.

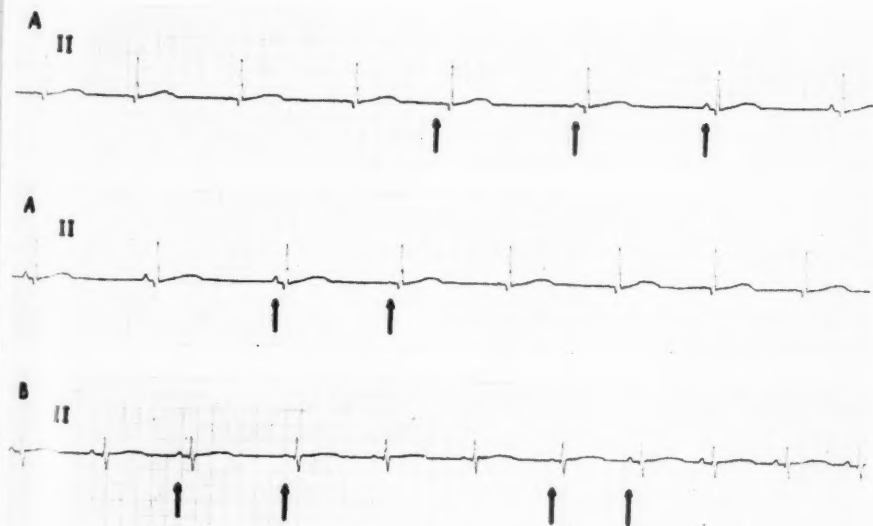


Fig. 6. Examples of supraventricular arrhythmia (lead II) in two babies (A and B) on the third day. The arrows mark change of rhythm.

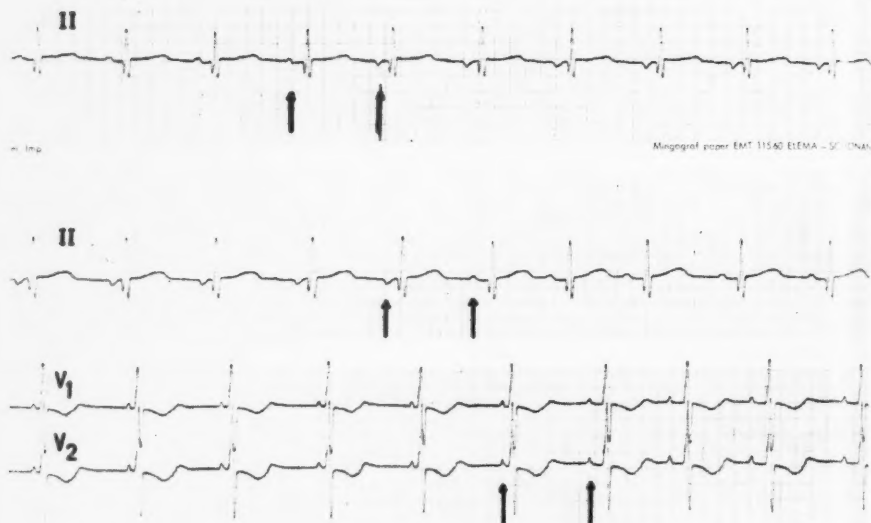


Fig. 7. Another example of supraventricular arrhythmia (on the fourth day). The arrows mark change of rhythm.

in such a way that with the lower heart rate, in lead II, the P wave was lower and shorter, and the P-R interval was also shorter. In three cases the P wave turned negative during the lower rate period. The differences in rate were 45 beats per minute at most. These arrhythmias were more common when lower heart rates appeared. In 7 of the 12 cases the changes in rhythm seemed to follow the wakefulness of the child in such a way that, during sleep, the lower rate dominated. The arrhythmias could, as a rule, be followed all through the recording time, and were often recorded on different days. They were observed at all ages but most often they occurred on the second and third day, and were gone in all cases but one on the seventh day. Examples of this type of ar-

rhythmia are given in Figs. 6 and 7. The electrocardiogram in Fig. 7 is of special interest as the periods with short P-R intervals in leads V1-2 seem to correspond to the periods with negative P wave in lead II. (These changes of rhythm occurred all through the recording time in lead II and V1-2 respectively. The leads were not recorded simultaneously, however.) Perhaps this could be taken as evidence that the supraventricular arrhythmias are of the ectopic, auricular type.

If the sinus arrhythmias are excluded, there are still 18 cases of arrhythmia out of 56 children. The long recording periods invalidate any comparison with other material. A considerably high frequency of arrhythmias in the first days of life seems to occur, contrary to earlier belief.

The P wave was measured in the first 100 beats of the recording. The P wave was measured in the first 100 beats of the recording. The P wave was measured in the first 100 beats of the recording.

The beginning of the recording was marked by a vertical line.

TABLE

Lead

I  
II  
III  
aVR  
aVL  
aVF  
V1  
V2  
V3  
V4  
V5  
V6

8-58

*P Wave*

The duration and the amplitude of the P wave were measured in all leads. The measurement in a few cases proved difficult in standard leads, owing to an after-potential partially confluent with the P wave. As a rule, the duration was the same in all leads. The longest duration noticed in any lead is reported. The mean value for the duration of P wave on the first day was 0.06, the minimum 0.05 and the maximum 0.09 seconds. The values for the seventh day were 0.05, 0.04 and 0.08 respectively. These figures are in agreement with those of Ziegler, except that we obtained higher values for the first 24 hours. There is also agreement as to the amplitude of the P wave, and these are presented in Table 1, where the supra-ventricular arrhythmias are excluded.

*P-R Interval*

The interval is measured from the beginning of the P wave to the beginning of the QRS complex. Even these measurements proved difficult in some cases, owing

to the after-potential. The longest P-R interval in any extremity lead is given. Table 2 shows the mean, minimum and maximum value of P-R interval in standard lead II correlated with the lowest rate measured in this lead. This rate is a mean value for six seconds. The supra-ventricular arrhythmias are not included. Contrary to other investigators, we found the P-R interval to be longer the first day. The explanation for this difference could be that in our material the first electrocardiogram has been registered such a short time after delivery. In fact all cases with P-R interval longer than 0.12 seconds were recorded within two hours after delivery.

*QRS Complex*

In no case did the ventricular activation time in the precordial leads exceed 0.03 seconds.

No Q wave was to be found in leads V1-2 in any patient.

The electrical axis of QRS was on the first day: mean 138, minimum 115, maxi-

TABLE 1. *The mean, minimum and maximum amplitude of the P wave of all cases, measured in  $\frac{1}{10}$  of a millivolt.*

Lead	First day of life			Seventh day of life		
	Mean	Min.	Max.	Mean	Min.	Max.
I	0.64	0.5	1.0	0.83	0.5	1.5
II	1.35	0	2.0	1.44	0	2.0
III	0.59	0	1.5	0.56	0	1.5
aVR	-1.04	-2.0	-1.0	-1.04	-2.0	-1.0
aVL	0.07	-1.0	0.5	0.25	0	1.0
aVF	0.90	0	1.5	0.97	0	2.0
V1	0.79	-1.0	1.5	0.80	0	1.5
V2	0.96	-1.0	2.0	1.05	0.5	2.0
V3	0.82	0.5	1.5	0.95	0.5	1.5
V4	0.86	0.5	1.5	1.00	0.5	2.0
V5	0.62	0	1.0	0.82	0.5	1.5
V6	0.70	0	1.0	0.88	0.5	1.5

TABLE 2. *The mean, minimum and maximum P-R interval in seconds in lead II according to age and minimum heart rate.*

Rate beats/min.	First day			No. of cases	Seventh day			No. of cases
	Mean	Min.	Max.		Mean	Min.	Max.	
80-100	0.10	0.08	0.12	7	0.09	0.07	0.10	6
105-120	0.11	0.08	0.14	27	0.09	0.07	0.12	21
125-140	0.12	0.08	0.14	21	0.10	0.09	0.11	18
145-160	0.11			1	0.10	0.09	0.12	11

mum 190 and on the seventh day: mean 136, minimum 110 and maximum 175.

The duration of the QRS interval, measured in seconds, is the same for the first and the seventh day, namely: mean 0.058, minimum 0.05 and maximum 0.07 seconds.

In Table 3 the amplitude of the R and S waves is given, comparing the values of the first and seventh day. Every value noted is a mean of three measurements. As a rule there were no measurable fluctuations of the amplitude during the recording and the difference never exceeded 2 mm. The comparison with the figures given by Ziegler shows that the values for

precordial leads, especially V1-3, are lower in our material. We cannot put forward any satisfactory explanation for this difference. If the size of the precordial electrode were significant, one would rather expect the amplitude in our material to be greater than in that of Ziegler. As far as one can understand, a few cases with very high amplitude are reported in Ziegler's work, and this could account for the discrepancy.

#### RS-T Segment

Depressions or elevations of this segment were measured in relation to a point located 0.04 seconds after RS-T junction

TABLE 3. *The mean, minimum and maximum amplitudes of the R and S waves (measured in millivolt) according to age.*

Lead	R wave first day			R wave seventh day			S wave first day			S wave seventh day		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
I	0.31	0	0.9	0.31	0	0.8	0.93	0.3	2.0	0.77	0.4	1.2
II	0.68	0.3	1.8	0.66	0	1.6	0.41	0	1.3	0.34	0	0.7
III	1.17	0	2.2	1.11	0.4	2.4	0.05	0	0.4	0.04	0	0.2
aVR	0.52	0	1.0	0.45	0.1	0.7	0.02	0	0.4	0.05	0	0.5
aVL	0.28	0	0.6	0.31	0	0.8	1.01	0.1	1.7	0.90	0.5	1.7
aVF	0.86	0.1	1.4	0.79	0.1	1.9	0.18	0	0.8	0.19	0	1.0
V1	1.48	0.5	3.3	1.33	0.5	2.6	0.88	0	3.0	0.75	0.1	1.8
V2	1.55	0.6	3.5	1.43	0.7	2.8	1.21	0.2	2.1	1.01	0.3	1.7
V3	1.56	0.6	2.6	1.41	0.5	2.3	1.81	0.4	3.6	1.40	0.7	2.6
V4	1.36	0.3	2.5	1.32	0.4	2.6	1.84	1.0	2.8	1.39	0.7	2.2
V5	0.79	0.2	2.3	0.92	0.1	2.0	1.47	0.6	2.5	1.24	0.4	2.4
V6	0.51	0.1	1.2	0.63	0.1	1.7	0.86	0.2	1.8	0.75	0.3	1.8

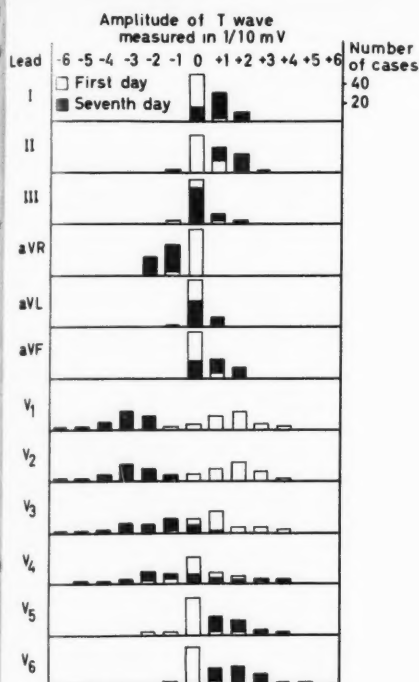


Fig. 8. Amplitude of T wave in all recordings on the first and seventh day.

as stated by Grewin (1948). The deviations in extremity leads never exceeded 0.1 mV. In the precordial leads there was as a rule a depression in V1-4, observed from the second or third day onwards, which never exceeded 0.3 mV.

### T Wave

The amplitude of the wave was measured in relation to the TP segment, at the lowest rate. There were measurable differences only with extreme variations of the rate, and the differences never exceeded 0.1 mV. In Fig. 8 the mean, minimum and maximum amplitude of all leads are reported, comparing the first and se-

venth day. The differences between the first and the seventh day in leads I, II, aVR, V1, V2, V5, V6 are highly significant ( $P < 0.001$  for each). This evolution of the T wave changes during the first week is well known and our results are essentially in accordance with earlier findings.

### U Wave

A U wave was identified in about  $\frac{1}{3}$  of the cases. It never exceeded 0.2 mV in amplitude and was as a rule detectable only with difficulty. The wave was not constantly occurring during a recording period.

### Q-T Interval

This measurement was sometimes complicated, owing to the U wave and the low T waves, and was performed according to Lepeschkin & Surawics (1952). The corrected Q-T interval ( $K$  in Bazett's formula) was in seven cases prolonged on the first day and in all cases within normal limits on the seventh day. When the Q-T ratio according to Goldberger (1948) and the Q-T time according to Ljung (1949) were calculated, it was found that the same seven cases with these methods also had a prolonged Q-T time on the first day and were all normalized on the seventh day.

### Comments

There are several findings in the electrocardiogram of the newborn, which at other ages should be called pathological, that must be considered to fall within normal limits. The most important of these findings are: Highly varying rates during short time-periods, with values as low as 70 and as high as 200 per minute.

Supraventricular arrhythmias. P-R intervals as short as 0.07 seconds. High R waves over the right heart, and deep S waves over the left heart. Extremely low T waves in standard extremity leads and augmented unipolar extremity leads. Positive T waves in lead V1 and negative T

waves in lead V6. Prolonged Q-T intervals during the first days.

It is of extreme importance to keep these facts in mind, when judging a case of suspect congenital heart disease, myocarditis, hypothyroidism or electrolyte disturbances.

### Summary

An electrocardiographic study was performed on 56 healthy children during their first week of life. Recordings were made repeatedly on every child using a long recording time. Leads I, II, III, aVR, aVL, aVF, V1, V2, V3, V4, V5, V6 were recorded.

Extreme fluctuations of the heart rate, a high frequency of sinus arrhythmia and supraventricular arrhythmia, signs of right ventricular hypertrophy, extremely low T waves with significant T wave changes during the first week and in some cases prolonged Q-T intervals were observed.

My thanks are due to Å. Gyllenswärd, M. D., for advice and criticism.

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## Motions of the Pharynx Associated with Initial Aeration of the Lungs of the Newborn Infant<sup>1</sup>

by JAMES F. BOSMA<sup>2</sup>, JOHN LIND and N. GENTZ<sup>3</sup>

As a part of a continuing study of initiation of respiration in the newborn infant, observations have been made of motions in the mouth, pharynx and larynx area by rapid sequential radiography. This region is found to be active prior to initiation of respiration and, in association with this event, a distinctive sequence of enlargement and diminution of the cavity of the pharynx has been noted. These pharyngeal area motions are distinguishable from swallowing of air into the esophagus and essentially resemble the distinctive glossopharyngeal inflation of the lung, similar to that described in poliomyelitis patients having severe motor respiratory deficiency and who have learned "frog breathing". More recently, repetitive glossopharyngeal inflation of the lung has been observed as an immediate sequel of paroxysmal pharyngeal and laryngeal closure (1).

### Methods

In the analysis of motions in the area of the pharynx and of the rapid aeration of the lung fields at birth, high speed roentgenographic technique is essential. In our exami-

nation we have employed a Schönander AOT changer capable of 6 exposures per second with exposure factors of 70-78 kV 200 ma 0.02 second.

The changer was placed at the foot of the labour bed and the mother was delivered in the supine position with her perineum at the end of the bed, so that the infant was in fact delivered direct on to the flat surface of the serial device. The newborn infant was placed upon its side and roentgen exposures were made at intervals of one half, one third or one sixth second beginning with the initial position time of place of the infant and continuing until respiration was visibly established. The roentgen ray was centered at the mid-thorax.

The possibility of excessive radiation of the infant was carefully considered and certain safeguards were employed.

The examinations were carried out with careful coning of the primary beam, and as far as possible the infant's gonads were shielded with lead rubber corresponding to 2 mm of lead.

Measurements carried out by the Radiophysiska Institutet, Stockholm (Radiophysics Institute), during the procedure gave the following skin doses.

<i>Schönander 24 × 30 cm films</i>	<i>Total dose</i>
15 exposures -560 Mr	0.6 r
30 exposures -1120 Mr	1.1 r

<sup>1</sup> This work has been supported by the Association for the Aid of Crippled Children, New York, U.S.A.

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The films obtained were compared visually and from selected films the pertinent structures were then traced upon transparent paper, using a consistent color key. These tracings could then be overlaid for evaluation of motions of small magnitude or of complex interrelations of motions. Intensity amplification of strategic items of the radiographs was accomplished by photographic means or by utilization of an electronic type of intensity amplification.

### Subjects

The infants under study in this project were born at term of normal pregnancy and delivery accomplished with minimal maternal sedation. The infants examined in this series were those who had not obviously respired while emerging from the perineum.

### Observations

This report is concerned primarily with the initial inflation of the lung and, accordingly, is restricted to eight cases, of the total of eleven roentgen series attempted, in whom initial inflation of the lung was obtained and the radiographs were technically satisfactory. In five of the eight there was a successive distention and compression of the pharynx in coincidence with this initial inflation of the lung. An example of this is given in figure 1. In four of these five the first inflation of the lung was found in coincidence with distention of the pharynx. In one of these five, this first inflation was seen with abrupt total closure of the pharynx.

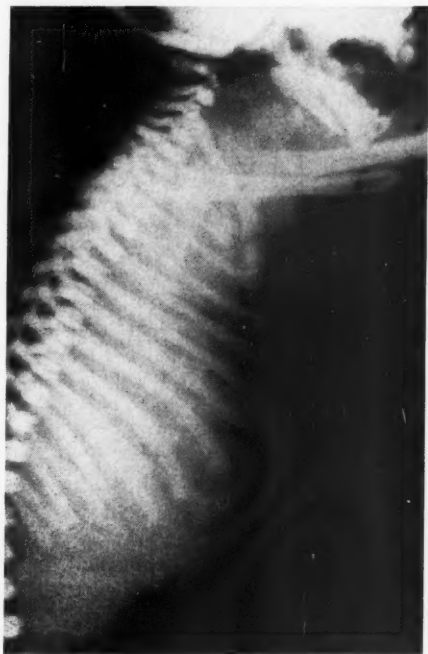
In one of the eight infants of the series, this study was terminated on the film showing the first inflation of the lung, thus no observation of a distention-compression sequence of the pharynx could be observed. In one of this series the distention-compression of the pharynx was in-

conclusive, as the pharynx was not observed to be entirely occluded. In the remaining one of the eight, initial inflation of the lung occurred without significant variation in pharynx diameters.

The distention phase of this sequence was the more variable in schedule and extent. It was identified by diminution in the interspace between the air-outlined posterior wall of the pharynx and the cervical vertebrae, by the breadth of the vallecular cleft between tongue and epiglottis, and by the relative position of the hyoid bone, as an indicator of the general position and motions of the ventral wall of the pharynx.

The occlusion of the pharynx was the more striking and the more significant. It was clearly distinctive from motions preceding the first air inflation of the lung or succeeding this (in three infants the roentgen observations were continued for more than 10 seconds following the first breath). The initiation of occlusion of the pharynx and larynx is in pattern identical with that of the "swallow" motions of glossopharyngeal breathing (1) in that there is a preliminary cephalad and dorsal displacement of the tongue toward palate. In two infants there is discernible a further gradation in a cephalo-caudal sequence of closure of the pharynx, with last traces of air discernible in the mesopharynx and laryngeal vestibule. At this stage, the trachea may be in undulating contour with a relatively broad antero-posterior diameter. At final occlusion the cavity of the pharynx and the vestibule of larynx are obliterated entirely, the hyoid is at its maximal elevation and the trachea is straightened. In none of these studies was air seen in the cervical esophagus. Re-

not ob. Fig. 1. Infant F. N., female infant, weight 3250 grams, length 50 cm, delivered at end of normal labor. Infant free of anomaly or apparent neurological impairment. Roentgenograms obtained at 3 per second. These examples are of successive radiographs.



1a. Prior to aeration of the pharynx. The contour of the neck and submental area is that found on cadaver examination of stillborn infants. The hyoid bone is cephalad to its normal rest position in the respiring infant.

opening of the pharynx and larynx occurs abruptly, without distinguishable gradations and, though expiration apparently follows promptly upon this re-opening, this is not associated with notable distention of pharynx.

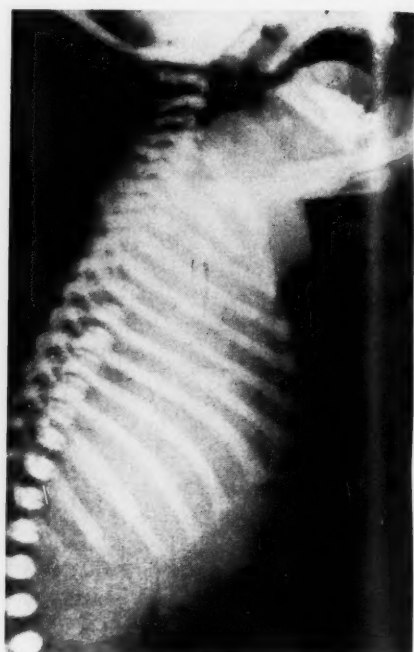
Prior to these motions of the mouth-pharynx-larynx area in association with initial air inflation of the lung, there were a variety of tongue motions, usually of gradation from a ventral protrusion with a pointed tip to dorsal-cephalic extension premonitory to swallow. These are in variable coincidence with air-filling of the

pharynx. In one infant the pharynx was twice filled and emptied actively in eight seconds and, after it was filled the third time, there was abrupt opisthotonic motions with distention of the pharynx and inflation of the lung. In the succeeding two seconds there was return to previous head-neck alignment coincident with progressive cephalo-caudal occlusion of the pharynx.

A particular effort was made to discern the abrupt opening and closing of the mouth described as a "snapping" by Schmidt (3) and by Ahlfeld (1888). This



1b. Initial air filling of the pharynx.



1c. Air now outlines the entire oral cavity and the epipharynx and mesopharynx.

was seen in one subject but, in the others, the maxillary-mandible relations were essentially unchanged. The motions of opening and closing of the mouth or of extension at the neck observed in this series were associated with filling of the pharynx rather than inflation of the lung.

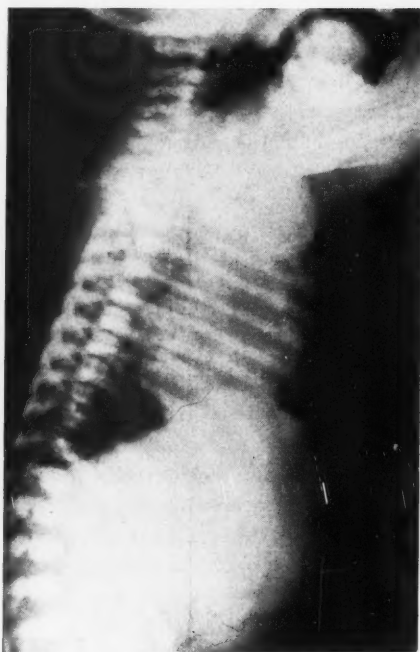
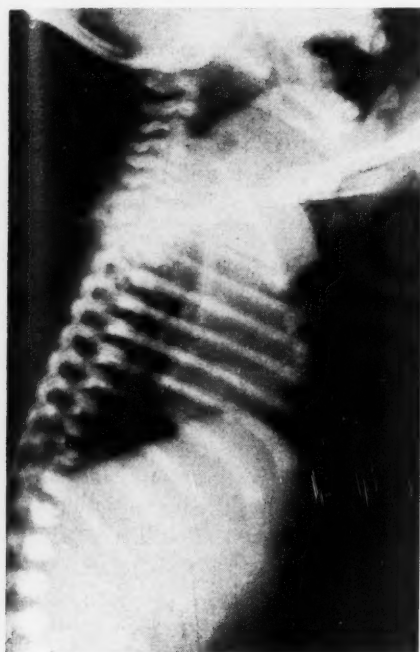
### Discussion

These observations by radiographs obtained at half- or third-second intervals give incomplete demonstration of the participation of the mouth, pharynx, and larynx in initial air inflation of the lungs. The performances of this region are characteristically rapid and complex. Their thorough evaluation depends upon cinefluoro-

graphy and other rapid radiographic methods. And continuous recordings of pressure and flow are required for elucidation of the physical effects of the motor performances of this series of valved cavities.

It is apparent from these radiographic observations, however, that the whole of the respiratory pathway is involved in initial air inflation of the lung and that this inflation does not occur after a period of motor immobility but against a background of organized performances of the pharyngeal region. And in the serial radiographs of one subject the successive air-filling of the pharynx itself appeared to be an active process.

The pharyngeal distention-compression



1d. The posterior portion of the tongue and the palate have moved dorso-cephalad and the tongue, palate, and posterior pharyngeal wall are in proximity. The hyoid has moved ventrad. The mandible is essentially unchanged in position. Increase in intrapharyngeal pressure is indicated by diminution of the retropharyngeal space. Air is present in the lung.

1e. The hyoid and mandible have moved slightly cephalad. The tongue, palate, and wall of the epipharynx are in tight closure. The posterior wall of the epipharynx is displaced farther ventrad than that of the mesopharynx. Air is present in the larynx and trachea (indicated by curved arrows). The diaphragm is further depressed and the epigastrium protruded.

sequence seen in five newborns at the time of first air inflation of the lung, is clearly distinguishable from these preliminary motions of the tongue and pharynx and also from lesser motions of this region associated with later respiration. The motions are also distinguishable from those of cry.

The specific physiological contribution of these pharynx area motions to initial inflation of the lung can only be inferred from these preliminary observations. It is apparent that the pharynx-larynx closure,

which is the more constant and distinctive element of these motions, prevents egress of air from the lung and may thus facilitate further distribution of air within the lung with increase in intrathoracic pressure, prior to the impending expiration.

The sequential motions of distention and compression of the pharynx are similar to those seen in glossopharyngeal respiration or "frog breathing" of respiratory-deficient poliomyelitis patients. Similar motions have been seen following focal pharyngeal seizures in an infant (1). From this

similarity it is suggested that the tongue and pharynx may actively inflate the lung. If so, this is probably supplementary to an immediately preceding thoracic inspiration, for both the lung and pharynx are found to be inflated abruptly in three of the subjects under study (see Fig. 1). Such a glossopharyngeal "breath" occurring exclusively or as a supplement to

thoracic inspiration would be strategic in accomplishing an instant of maximal initial pressure by which to overcome the cohesive force and other elements limiting primary expansion of the lung. This would thus be a physiological prototype of the "puff" methods of initiation of inflation of the lungs (2, 4).

### Summary

In five of eight subjects satisfactorily observed by successive radiographs within the immediate newborn period, the initial air inflation of the lung was found to be associated with a sequential distention and compression of the pharynx cavity. It is acknowledged that these observations are preliminary and incomplete. But a similarity is recognized to the glossopharyngeal breathing reported in respiratory-deficient poliomyelitic subjects and also a part of patterns of manifestation of focal seizures in the pharynx area of an infant.

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## Limitation of Movement of the Hip Joints in New-born Infants Following Breech Presentation

by GILLIS HERLITZ

After frank breech presentations, in which the legs are extended and lie against the abdomen and thorax, a characteristic defect of movement of the hips is common, and may persist for several weeks or even longer. This type of breech presentation is the commonest of the usual forms. In the complete breech, the hip and knee joints are in extreme flexion, and the feet are born at about the same moment as the breech. This type of presentation may also be followed by limitation of movement of the hips, but apparently less commonly than the breech with extended legs.

The defect of movement becomes evident during the first days of life, as a characteristic attitude of the legs, which will often at a single glance indicate that the child was born by the breech. This sign seems to have been observed by many experienced midwives, but I have been unable to find any description of it in current text-books of obstetrics or in the paediatric literature. A brief account of the phenomenon therefore seems to be justified.

When the infant is lying supine, the hips will typically be flexed at an angle of 55–60°, with only slight abduction and

external rotation. The knees are flexed at an angle of about 135°, and the angle at the ankle is roughly similar. See Figs. 1 and 2. The normal position of the legs in the new-born is as follows. The hips are more markedly flexed, abducted, and externally rotated, and the knees are flexed, so that the legs and feet are crossed. Tests of passive movement of the hips in infants with the defect above described show that rotation, flexion, and especially abduction and extension are greatly impaired. Flexion at the knee and ankle joints is also limited. The limitation of movement diminishes gradually during the course of the next few days, but the impairment of abduction and flexion of the hips may persist for several weeks. In one of my cases obvious limitation of extension and abduction was still demonstrable in both hips, even after three months, but this later disappeared.

Radiography of the hip joints, which I have had done in 13 typical cases, will sometimes reveal distension of the joint capsule. No increase in the fluid of the knee joint has been demonstrable. No dislocation or subluxation of the hip was present in any of the cases, and no trau-

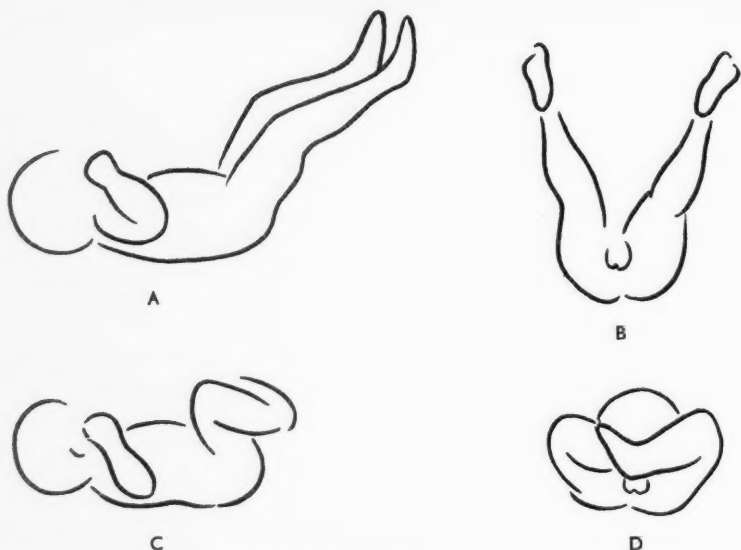


Fig. 1. *A, B.* The abnormal attitude of the legs described. *C, D.* The normal position of the legs in the new-born.

matic separation of the upper femoral epiphysis. This last-named injury is said sometimes to occur during complete breech extraction owing to violent traction on the thigh, together with rotation and marked abduction (Ehrenfest), and the clinical picture is closely reminiscent of that in

congenital dislocation of the hip, which is not caused by trauma.

It is clear that the abnormal attitude of the legs and limitations of movement described reflect the position of the legs during birth, and are a direct result of this. In frank breech presentations the obstetrician



Fig. 2. New-born girl with the abnormal attitude of the legs described.

sometimes has to insert one index finger and apply traction on the groin, and then, when the breech is born, continues traction downwards and forwards with the other index finger in the gluteal fold, the trunk subsequently being directed upwards towards the mother's abdomen. A blunt hook is seldom applied in the case of a living child, and was not employed in any of those above-named. It is of course possible that digital traction on the groin may have contributed to the distension of the joint capsule observed in some of the cases, but it cannot have been of any great importance in causing the abnormal attitude of the legs and limitation of movement of the hips. The characteristic limitation of movement was present even in those cases of frank breech which were delivered without interference.

Investigation of a series of about 40 cases of frank breech revealed that the incidence of limitation of movement as described is commoner than it might be

believed. About half of the cases showed striking, typical malposition of the legs, and in a further quarter of the series limitation of passive extension and abduction at the hip joints (the latter tested with the infant lying supine and the thigh at a right angle to the horizontal plane) was easily demonstrable during the first few days of life.

The clinical significance of the abnormality is that it may be interpreted as a spastic state, congenital dislocation, or a more serious birth injury. Treatment will generally be unnecessary, but the nursing staff should be instructed to exercise care in handling the child, especially when changing napkins and clothes, and to place the child on its side in the cot, as the weight of the bedclothes may exert undue pressure on the legs, thus damaging the hip joints which are temporarily immobilized owing to increase in intra-articular fluid, swelling of the joint capsule, and peri-articular oedema.

### Summary

A description is made of a common, characteristic defect of movement of the hip joints during the neonatal period and the next few weeks, following breech presentation.

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## Renal Biopsy in Children

by C. G. BERGSTRAND and H. BUCHT

Renal biopsy has in recent years developed into a method of great value in the study of renal diseases. It makes it possible to correlate the clinical and laboratory findings with the actual pathological status of the kidney and may in selected cases be an important diagnostic and prognostic aid.

Renal biopsy has been used relatively infrequently in children. This is probably due to the fact that the method is supposed to demand a certain cooperation on the part of the patient, which at least in smaller children is not always easily obtained.

The purpose of this paper is to present the technique of renal biopsy as practised at Kronprinsessan Lovisas Hospital for Sick Children.

The method is essentially the same as that used in adults for several years by Bucht. It has been described in detail by Kark & Muerhcke (1954).

Before biopsy a radiogram is taken to determine the position of the kidneys. The patient should preferably be in the prone position but this is not absolutely necessary. Radiograms taken with the patient on his back have been proven to give a correct localisation. It is perhaps more essential that the radiogram should not be taken in deep inspiration or expiration.

The day before the biopsy a blood count including thrombocytes is made. Clotting time, bleeding time, the Quick index and the patient's blood group are determined.

Thirty minutes before the biopsy the patient is given morphine-scopolamine subcutaneously. In older children it is then quite possible to make the biopsy under local anesthesia but in younger or nervous children a short general anesthesia is preferable. The patient is placed in the prone position with a hard cushion under the abdomen on a low table. The lower part of the kidney is generally chosen for the biopsy, and the place on the skin, where the needle is to be inserted, is determined from measurements taken from the radiogram as described by Kark & Muerhcke (1954). Experience has proved that it is quite possible to control the position of the needle from the movements made by the kidney with respiration even when the patient is under general anesthesia. The characteristic swinging of the needle is, however, admittedly easier to observe, when the patient is awake and can be told to breath deeply.

The biopsy is made with the same needle used in adults (Vim-Silverman needle as modified by Franklin). It is essential that the needle is fitted with the "he-is"

described by Franklin to get a satisfactory specimen.

After the biopsy, which usually is performed in the morning, pulse and blood pressure are controlled every second hour three to four times.

Renal biopsy has been made in 17 patients 4 to 16 years old. The clinical diagnoses are given in the table. No severe complications have been observed. The haematuria which follows a renal biopsy has usually been microscopic and of a few days duration. In one patient a more massive bleeding was observed for a few days but the patient did not need blood transfusion or special treatment. When haematuria existed before the biopsy, an increased number of red cells could be seen in the sediment.

In all patients it has been possible to obtain a satisfactory specimen of renal tissue for histological examination. In one patient, however, the biopsy was not successful the first time but was repeated a week later with good results. In some cases a part of the specimen has been examined by electron microscope. These studies will be published later in collaboration with A. Bergstrand. In one case of suspected pyelonephritis a culture was made from the specimen. The result was

TABLE 1. *Diagnosis and number of patients subjected to renal biopsy.*

Diagnosis	No. of patients
Haematuria of unknown origin	3
Atypical acute glomerulonephritis	6
"Purpura nephritis"	4
Nephrotic syndrome	1
Miscellaneous cases with suspected renal disease	3
Total number of patients	17

negative and the general impression from our experiences with adults is that renal biopsy specimens seldom give positive cultures even in advanced cases of pyelonephritis.

The relatively small number of children who have been subjected to renal biopsy does not allow of too wide conclusions being drawn regarding the practical value of the method in this age group. The experience has, however, so far been encouraging. In several cases, where the clinical diagnosis could not be definitely established, valuable information has been obtained by histological examination. It has been possible to confirm a suspected clinical diagnosis or to exclude more severe renal lesions.

### Summary

The technique of renal biopsy in children is described and the clinical value of the method is pointed out.

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## Some Physical Measurements (Weight, Length, Head Circumference and Chest Circumference) in Healthy Swedish Children in the First Two Years of Life<sup>1</sup>

by PETTER KARLBERG and ALICE PERMAN

Weight and anthropological measurements have been considered valuable factors in the judgement of growth and development of children. However, there are few standards of physical measurements available for children up to 2 years of age in Sweden.

The first publication in this field, now mainly of historical interest, is V. O. Peterson's (6) report on a follow-up study of the weight in 13 infants during the first 10–11 months of life. Höjer (4) in 1925 published standards for weight during the first year of life, derived from data obtained from children's welfare clinics in a suburban district of Stockholm and collected during the years 1920–1924. v. Sydow (9) in 1941 published an extensive study of the weight of healthy breast-fed infants during the first year of life, born in Gothenburg during the years 1927–1937. He showed the influence of birth weight on the physical growth. Broman, Dahlberg & Lichtenstein (1) in 1942 gave standards of weight and height in Swedish children aged 1–20 years, measured during the years 1938–1939. These standards are

those most often used for older children in Sweden. However, the data from the second year of life were divided into two groups only, with the class middle of 15 and 21 months and the numbers of cases in these groups fairly small.

The aim of the present investigation is to give standards of weight, length, head circumference and chest circumference in Swedish children during the first two years of life, derived from the same material.

### Material and Methods

The measurements were made in two periods, March–October 1950 and January–March 1952. During the first period we tried to measure the children close to an exact age in terms of whole months, from 1–12 months. For children more than one year of age, we concentrated the measurements in children aged 15, 18, 21 and 24 months. However, sometimes we included children in the ages between. Some children were measured several times, but most of them only once.

The survey was continued in 1952 after an interval of about one year. One reason was to increase the material and another was to get the birth months of the cases in

<sup>1</sup> The study was made possible by a grant from Stille-Werner Co, Stockholm.

TABLE 1. *Sampling.*

Study period...	Recruited cases		Rejected cases according to medical or improper examina- social reasons tion date				Analysed cases		
	1950	1952	1950	1952	1950	1952	1950	1952	Total
Boys	357	492	15	30	12	91	330	371	701
Girls	335	473	16	30	11	91	308	352	660
Total	692	965	31	60	23	182	638	723	1361

each age group well spread throughout the year. During this time each child was measured only once, but children with less strict limits regarding the age group were included. The survey was concluded when we estimated having recruited 50 boys and 50 girls in each month group from 1-12 months, and at least the same number at every 3-month interval in the ages over one year.

The measurements were made at nine welfare clinics in the southern suburbs of Stockholm (Hökarängen: west, east, north, south; Enskede: east, west, south; Johanneshov: east, west). The residential areas in these welfare clinic districts developed during 1946-1951, and mostly consisted of modern apartment houses, including some one-family houses. Some of these apartment houses were constructed rapidly and not too well, as an emergency housing measure. Because these districts included a large variety of housing conditions, a wide range of economic standards was included in this study. The population in these areas consisted in the main of young families most of whom had moved from either the center of Stockholm or from rural areas. The ratio between these groups was about one to one. All different social groups were represented in this population. 91% of all children under one year of age were registered at welfare clinics.

#### Recruitment

Children visiting the welfare clinics at the time of the survey were measured. If a large number of children fell in the age group up to one year, the measurements

were performed on those who met the requirements of being inside the limits of  $\pm 7$  days for an exact month. All older children were measured as they visited the welfare clinics at larger intervals. Otherwise there was no specific selection.

In this way we recruited 787 boys and 726 girls, see Table 1. In order to increase the number of children in the age group 1-2 years, 62 boys and 82 girls in day nurseries were measured during 1950.

#### Measurements

All measurements were taken by one person, a well-trained and experienced public health nurse, the same on both occasions. The techniques of measurements were tested with the authors before the collection of data was started.

For the weight measurements we used the same basket scale (periodically checked), for distance measurements a flexible, narrow-width steel tape was used.

The following measurements were made:

*Weight.* All children were weighed naked to the nearest 10 grams.

*Length.* The recumbent length was measured in all children. The child was lying on a table with its short end against the wall. The head of the child touched the wall, both legs were stretched out and the feet were resting against a movable wood panel, angled perpendicular to the table surface and parallel to the wall. The distance between the wall and the panel was measured. In children who could walk and stand erect, the standing height was measured.

The child leaned against the wall and the same measuring panel was used, now in right angle in relation to the wall. 193 such measurements were performed.

**Head circumference.** This was taken at the maximum circumference, with the tape passing round the occipital and frontal areas and held perpendicular to the mid-sagittal plane.

**Chest circumference.** This was taken at the nipple level.

During 1950 nine more measurements were made: the circumference of neck, waist, hip, upper arm, thigh and ankle; and the length of the trunk, arm and leg. These measurements will not be presented in this paper.

#### *Sampling of the recruited children*

Only data of healthy children were planned to be analyzed for these standards. About six months after each concluded observation period the thoroughly kept records from the welfare clinics were examined, giving us a longitudinal follow up. We eliminated all children with evidence of acute or chronic disease during the observation period, as well as children with suspected inadequate nutritional states and inappropriate social environment. Children were also eliminated if both parents were not born in Sweden. If the total number of children measured, 45 boys and 46 girls were eliminated for medical or social reasons, as shown in Table 1.

In the age groups 1-8 months those children were selected who were examined within  $\pm 7$  days to a whole month. Thus 103 boys and 102 girls were sorted out (see Table 1). As the influence of the variation in the age within an age group decreases with increasing age, no such selection was made after 9 months of age, i.e. a variation of  $\pm 15$  days was accepted.

The total number of children from which data will be presented in this paper were 1361 (701 boys and 660 girls).

A selection regarding the birth weight was not made. In the 481 children measured

before 9 months of age, the birth weight was distributed as follows:

<i>Birth weight in kg:</i>					
< 2	2.00-2.49	2.50-4.49	4.50-4.99	> 5.00	
<i>Number of cases:</i>					
0	7	469	5	0	

#### **Results:**

The results have been grouped in relation to age. Table 2 shows the frequency of measurements in each month group for boys and girls in the material from both 1950 and 1952. The number of longitudinal data from 1952 is also presented. The significance of a difference between the two materials (1950 and 1952) has been tested by the *t*-test (7) using a calculated mean with the mean error in each group for both sexes for the four body measurements respectively. Very few of all the pairs of corresponding means showed a significant difference and the difference did not show any systematical trend. Thus the two materials were mixed and have been treated as one.

The mean and standard deviation (S.D.) of length, weight, head circumference and chest circumference have been calculated separately for boys and girls in each age group in the mixed material given in Table 3. From 1-12 months, each month has been used as an age group. After 12 months, class intervals of 3 months have been used, as the growth velocity decreases with increasing age. As 15, 18 and 21 months were selected as class middle, data from 13, 23 and 24 months of age were excluded. Data from the two last groups could not represent an age group of 24 months, as there were no values for 25

TABLE 2. *Age and sex distributions.*

Material...	Boys				Girls			
	1950		1952	1950 + 1952	1950		1952	1950 + 1952
	No. of cases...	330	100	371	701	308	78	352
Age in months	Cross-sect. data	Longit. data	Cross-sect. data	Total no. of data	Cross-sect. data	Longit. data	Cross-sect. data	Total no. of data
1	33	—	11	44	25	—	11	36
2	7	23	23	53	7	20	26	53
3	18	19	11	48	15	19	15	49
4	5	26	18	49	6	21	18	45
5	6	27	15	48	13	21	16	50
6	20	22	8	50	18	22	22	62
7	9	21	14	44	6	27	19	52
8	15	16	17	48	10	18	14	42
9	28	18	34	80	17	18	25	54
10	9	22	26	57	11	18	27	56
11	17	22	24	63	7	15	15	37
12	22	22	22	66	20	10	24	54
13	16	6	16	38	11	—	7	18
14	4	1	16	21	11	—	9	20
15	21	8	17	46	22	1	13	36
16	8	—	11	19	14	—	11	25
17	14	—	18	32	14	—	10	24
18	25	2	14	41	19	2	9	30
19	9	—	13	22	9	—	15	24
20	9	1	10	20	13	—	8	21
21	8	1	9	18	14	2	13	29
22	5	—	12	17	10	—	10	20
23	9	—	5	14	10	—	9	19
24	13	—	7	20	12	1	6	19
Total	330	257	371	952	308	215	352	875

months. In each age group for the respective body measurements the significance of sex difference is tested by the *t*-test (see Table 3). The boys show systematically significant higher values.

The collected data have also been grouped in relation to length in a class interval of 5 cm. In this analysis only the cross-sectional data were used to avoid having more than one data for each individual included in the same length group. For each of the three body measurements—weight, head circumference, and chest circumference—the sex differences within

each length group have been tested by the *t*-test, see Table 4. These sex differences of the length groups are of a much smaller magnitude than in the age groups, and the significance of the differences is definitely less frequent. Thus the data of the boys and girls are mixed and the means and S. D.'s of the three body measurements are given (in Table 4) for each length group. The difference between recumbent length and standing height was calculated in 193 cases. The mean difference was 1.5 cm with a S.D. of  $\pm 1.9$  cm (mean error  $\pm 0.13$  cm).

TABLE 3. Length, weight, head circumference and chest circumference in relation to age and sex.

Age groups in months			Length in cm			Weight in kg			Head circumference in cm			Chest circumference in cm		
Class middle	Sex <sup>a</sup>	No.	Mean	S.D.	Sex diff. <sup>b</sup>	Mean	S.D.	Sex diff. <sup>b</sup>	Mean	S.D.	Sex diff. <sup>b</sup>	Mean	S.D.	Sex diff. <sup>b</sup>
1	B	44	54.3	2.1		4.07	0.49		37.6	1.1		36.2	1.8	
	G	36	53.7	1.8	+0.58	3.95	0.59	+0.12	37.1	1.1	+0.53*	35.9	1.8	+0.29
2	B	53	57.4	2.6		5.01	0.62		39.5	1.0		38.2	1.5	
	G	53	56.1	2.5	+1.26*	4.56	0.65	+0.45**	38.4	1.2	+1.13**	37.2	1.7	+1.03**
3	B	48	61.0	2.1		5.85	0.60		40.7	1.2		40.2	1.5	
	G	49	60.2	1.8	+0.78	5.60	0.56	+0.25*	40.0	1.3	+0.64*	39.6	1.7	+1.64**
4	B	49	63.5	2.2		6.50	0.72		41.8	1.3		41.4	1.8	
	G	45	61.8	1.8	+1.74**	6.06	0.60	+0.44**	40.8	1.1	+1.00**	40.6	1.8	+0.75*
5	B	48	66.3	2.2		6.99	0.72		42.8	1.2		42.2	1.8	
	G	50	64.5	2.0	+1.81**	6.70	0.62	+0.29*	41.7	1.0	+1.11**	41.9	1.8	+0.25
6	B	50	68.2	2.2		7.69	0.81		43.8	1.1		43.9	2.1	
	G	62	66.5	2.3	+1.71**	7.29	0.68	+0.40**	42.9	1.0	+0.91**	43.1	1.9	+0.78*
7	B	44	69.7	2.5		8.40	0.98		44.8	1.1		45.0	1.9	
	G	52	68.4	2.3	+1.33**	7.71	0.85	+0.69**	43.6	1.1	+1.13**	44.0	1.7	+1.79**
8	B	48	71.8	2.4		8.91	0.86		45.3	1.4		45.8	2.2	
	G	42	69.4	2.5	+2.46**	8.16	0.88	+0.75**	44.0	1.0	+1.30**	44.8	1.8	+1.03*
9	B	80	72.4	2.5		9.30	1.10		46.2	1.6		46.9	2.2	
	G	54	70.7	2.6	+1.63**	8.57	0.88	+0.73**	44.9	1.1	+1.34**	45.6	2.2	+1.38**
10	B	57	73.6	2.6		9.43	0.90		46.2	1.2		47.0	1.9	
	G	56	72.0	2.2	+1.58**	9.04	0.96	+0.39**	45.3	1.2	+0.90**	46.4	2.2	+0.60
11	B	63	74.6	2.1		9.79	1.01		47.2	1.4		47.8	1.9	
	G	37	74.1	2.6	+0.49	9.55	1.09	+0.24	46.2	1.2	+1.06**	47.1	2.3	+0.69
12	B	66	75.4	2.5		10.02	1.03		47.3	1.4		48.3	1.8	
	G	54	74.0	2.7	+1.98**	9.65	1.05	+0.38*	46.3	1.2	+1.05**	47.3	2.4	+1.04**
15 (14-16)	B	86	78.4	2.4		11.00	1.02		48.3	1.3		49.3	1.9	
	G	81	77.4	2.9	+0.94*	10.53	1.17	+0.47**	48.1	2.0	+0.21	48.3	2.3	+1.02**
18 (17-19)	B	95	81.9	3.1		11.85	1.15		49.0	1.3		50.2	2.2	
	G	98	81.0	3.1	+0.90	11.14	1.20	+0.71**	48.6	1.7	+0.40	48.8	1.9	+1.41**
21 (20-21)	B	55	84.6	2.7		12.34	1.63		49.4	1.3		51.1	1.8	
	G	49	83.9	3.4	+0.70	11.82	1.32	+0.52*	49.1	2.0	+0.31	49.5	2.0	+1.55**

Length  
Interval  
classes

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50.0-5

55.0-5

60.0-6

65.0-6

70.0-7

75.0-7

80.0-8

85.0-8

90.0-9

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TABLE 4. *Weight, head circumference and chest circumference in relation to length and sex.*

Length groups in cm				Weight in kg			Head circumference in cm			Chest circumference in cm		
Interval classes	Sex <sup>a</sup>	No.		Sex diff. <sup>b</sup>	Mean	S.D.	Sex diff. <sup>b</sup>	Mean	S.D.	Sex diff. <sup>b</sup>	Mean	S.D.
45.0-49.9	B G	1 2	3	—	—	—	—	—	—	—	—	—
50.0-54.9	B G	34 36	70	+ 0.03	3.84	0.38	+ 0.38	37.4	1.1	- 0.11	35.6	1.3
55.0-59.9	B G	45 46	91	- 0.08	4.87	0.55	0.00	39.2	1.3	- 0.07	38.2	1.7
60.0-64.9	B G	49 69	118	- 0.20*	6.20	0.60	+ 0.44	41.4	1.4	- 0.32	40.8	1.6
65.0-69.9	B G	80 100	180	+ 0.12*	7.62	0.77	+ 0.45	43.8	1.3	+ 0.27	43.8	1.9
70.0-74.9	B G	146 127	273	+ 0.13**	9.15	0.85	+ 0.50*	46.0	1.5	+ 0.23	46.6	2.0
75.0-79.9	B G	162 115	277	+ 0.01	10.49	0.86	+ 0.86**	47.6	1.4	+ 0.55	48.6	1.9
80.0-84.9	B G	117 109	226	+ 0.03	11.71	0.92	+ 0.99**	48.7	1.3	+ 1.25**	49.8	2.0
85.0-89.9	B G	62 50	112	+ 0.03	12.87	0.94	+ 1.01**	49.4	1.2	+ 1.05*	51.0	1.9
90.0-94.9	B G	5 6	11	—	(13.55)	(0.84)	—	(49.7)	(1.0)	—	(51.6)	(2.0)

<sup>a</sup> B = boys; G = girls.<sup>b</sup> Sex difference = (mean of boys - mean of girls). The probability of the two means samples belonging to the same group is marked \* when between 0.05-0.01 and marked \*\* when < 0.01.

### Comments

The sample from which the collected data are drawn represents healthy Swedish children living in modern districts originating from a population moved in from urban and rural areas. The data are predominantly cross-sectional. The longitudinal data have only been used for the age grouping. Within each group, the data from one case is only presented once.

The mean values presented for weight and length in the different age groups are graphically compared (Figs. 1 and 2) with

similar values from earlier investigations. There is a good conformity with v. Sydow's (9) values for weight in children with a birth weight of 3.0-3.5 kg, representing the first 12 months of life. Height measurements were not presented in his survey. However, within the overlapping age range (15 and 21 months) the material of Broman, Dahlberg & Lichtenstein (1) shows higher values, especially in weight. The differences are more marked in the age group of 15 months than those of 21 months. Compared with two American

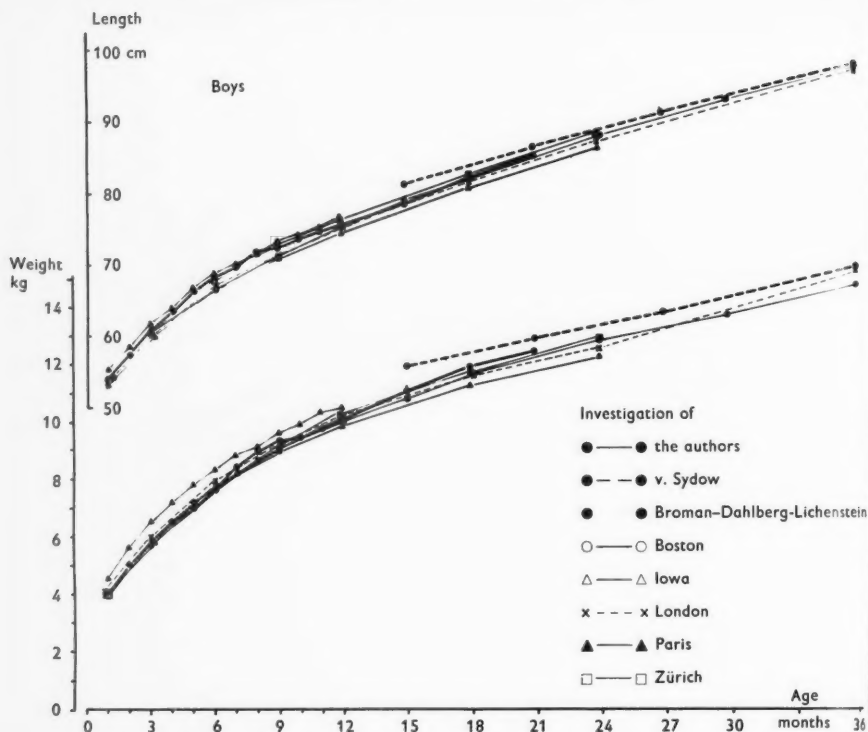


Fig. 1. Boys. A graphical comparison of standards of length and weight obtained from studies in boys (investigations of v. Sydow (9), Broman, Dahlberg & Lichtenstein (1), Boston (8), Iowa (5), London (2), Paris (3), Zürich (3)).

(5, 8), one English (2), one French (3), and one Swiss (3) investigations, our values show a good general agreement throughout the whole age range. However, the Iowa study shows a tendency to higher figures but is clearly in better agreement with our studies than the one of Broman, Dahlberg & Lichtenstein. The reason for the high values in the latter may be the relatively small numbers of their cases in the two lowest age groups, 15 and 21 months (32 and 37 boys respectively, and 38 and 33 girls respectively). Each age

group has also a wider range, 6 months, than those of our study, 3 months.

In judging a child's growth, the body height in relation to age is used primarily. Since this relationship is normally distributed, the use of the mean and S.D. is adequate. In judging the nutritional state of a child, the weight is often correlated to the age. However, a tall, slim individual can weigh as much as a short, fat one, as the weight also reflects the height. The judgement of the nutritional state will therefore be more adequate when the

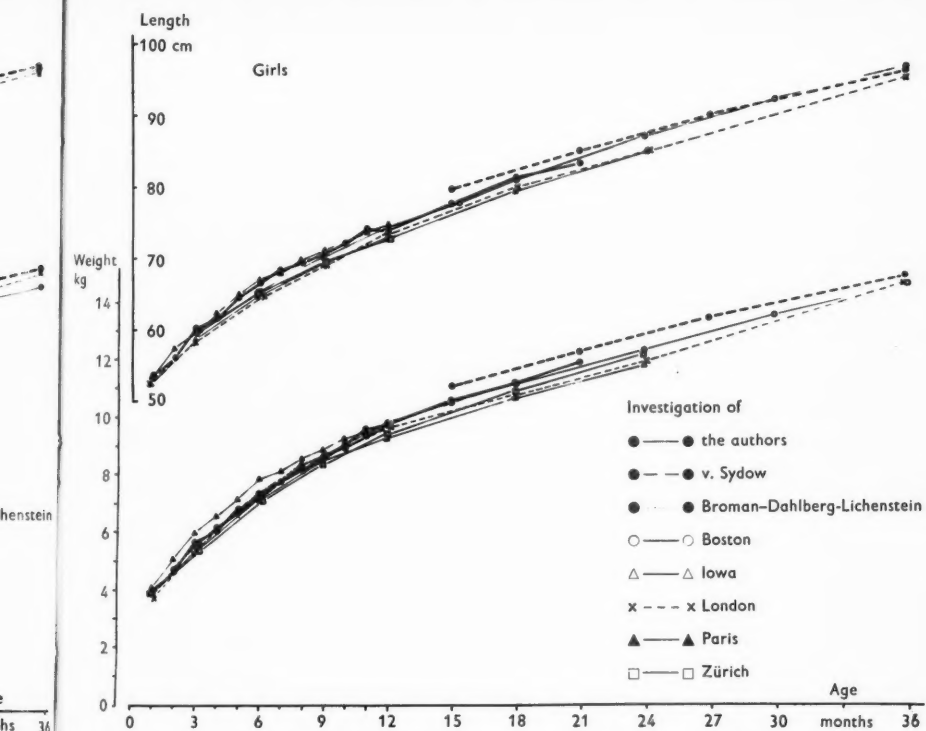


Fig. 2. Girls. A graphical comparison of standards of length and weight obtained from studies in girls (investigations of v. Sydow (9), Broman, Dahlberg & Lichtenstein (1), Boston (8), Iowa (5), London (2), Paris (3), Zürich (3)).

weight is correlated to height. The sex differences also diminish.

This relationship does not show any clear trend to asymmetrical distribution in this material as plotted, while the relationship weight/age shows this trend to some extent. However, even in the analysis of this relationship, mean and S.D., and not percentiles, have been calculated because this study was primarily performed in order to complete the standard values of Broman, Dahlberg & Lichtenstein (1), and these are given in means and

S.D.'s. It is shown that the standing height is shorter than the recumbent length and this is in conformity with other investigations (2).

The head circumference and the chest circumference represent two prominent body dimensions and they are also related to age. If in the judgement of separate cases we wish to decrease the influence of individual velocity of growth, the relation to another body measurement is adequate. For the head circumference the height is more adequate than the weight, and the



influence of rapid nutritional changes is thus avoided. Also the chest circumference is related to body height. Using these relationships there are decreased sex differences in both circumference measurements.

### Summary

Weight (naked), recumbent length, head circumference and chest circumference have been measured in 1361 healthy Swedish children (701 boys and 660 girls) within the age range of 1 to 24 months. The children were born in the period 1948-1951 and their parents came from both urban and rural populations and lived in modern areas of Stockholm.

Means and standard deviations of the four body measurements are given for different ages. With the material grouped according to age there are significant systematical sex differences, hence the means and the Standard deviations are given separately for boys and girls. When grouped in length classes, the weight, head circumference and chest circumference show less marked sex differences, so means and standard deviations are given together for boys and girls.

A graphical comparison with some earlier investigations in Sweden and other countries is presented.

Our data indicate that in clinically evaluating the growth of a child, the length should be correlated to age, and the weight correlated to length. Head circumference and chest circumference should also be correlated to another body measurement.

We are grateful to Miss Zita Olsson, R.N., who performed the measurements faithfully during the two periods of study. Our thanks are also due to the nurses of the welfare clinic whose co-operation was invaluable.

### Appendix: A Swedish Growth Chart

(With the collaboration of STEN IGGBOM)

A combination of the standards presented for ages up to 2 years and the standards of Broman, Dahlberg & Lichtenstein for the ages 1-20 years now gives the opportunity to judge physical growth and development in Swedish children regarding height and weight from 1 month up to adolescence. In order to simplify the use of these standards in the judgement of individual cases, a Growth Chart has been worked out, see Fig. 3.

The chart was constructed to contain the following conditions: Height in rela-

tion to age; weight in relation to height; separate values for boys and girls in the age groups with systematic significant sex differences of clinical importance; head circumference in relation to height range of the first two years of life; the variations around each mean in 95 % confidence interval; the same chart applicable for both boys and girls, at least covering the pre-school ages.

*Comments:* The 95 % confidence interval around each mean (i.e. approximately  $\pm 2$  S.D.) was selected because the limits representing 2.5 % probability of belonging to a normal population are considered to be clinically most useful. By using a

99 % confidence interval (i.e. approximately  $\pm 2.5$  S.D.), 0.5 % probability limits will be obtained and these have been considered to be too large for clinical application. The differences between the two standards within the overlapping age period (15–21 months) have been smoothed out, giving the Karlberg–Perman standards more credulance. Logarithmic scales have been used in order to cover appropriately a wide age range in the same chart. The contiguous means and also the contiguous 95 % confidence limits

are connected with a smoothed curve in order to simplify the interpolation between values. The age scale through which the chart is entered, is placed to the right, as a convenience for right-handed persons.

The chart has been used in the records of the pediatric clinic at Karolinska Sjukhuset during the last 3 years and has been found quite useful.

Summary: A Swedish Growth Chart is presented, predominantly designed for the evaluation of preschool children.

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## The Intravenous Administration of Species-specific Milk

### Some Experiments on Animals

by OLOF MELLANDER, GERDA NILSSON and STURE HEDENSTEDT

The direct intravenous administration of non species-specific proteins is not as a rule possible owing to the antigenic qualities of these substances, the use of species- and group-specific blood plasma having therefore been necessary. The readily available source of protein, which milk constitutes, does not seem to have been used in intravenous nutrition, however, but there are several reasons justifying detailed experiments with species-specific milk for this purpose. Thus it has quite recently been shown (Hansson & Johansson) that several proteins in human milk are immunologically identical with proteins occurring in blood serum: one of the proteins present in milk corresponds to the serum albumin; and the same seems to be true also of other electrophoretic fractions such as the  $\gamma$ -globulin. Experiments carried out so far do not exclude the possibility that identity can exist between other blood and milk protein fractions. The most characteristic protein of the milk, casein, shows relatively weak antigenic properties and casein from the milk of different species cannot be distinguished in experiments on sensitized guinea-pigs, although

there are very pronounced chemical and biochemical differences. Species-specific milk proteins have not shown any antigenic properties in experiments performed so far. There is reason to assume (Mellander, 1947, 1955) that the proteins in milk can be utilized without having undergone complete hydrolysis. In hydrolysis experiments performed in vivo and in vitro, the proteins of human milk have shown a striking resistance to proteolytic enzymes. The existence of trypsin inhibitors in both bovine and human colostrum must also act as a check on intestinal hydrolysis. There are, then, several factors indicating that at least some of the protein substances of milk are absorbed in the gut in a more or less unchanged state, at any rate during the first period of life. There is therefore the intriguing possibility of using species-specific proteins when the need arises for introducing proteins intravenously for purpose of nutrition. In addition milk contains other valuable nutrients such as carbohydrates and fat. Of these, fat might constitute a source of trouble but, as our preliminary experiments have shown, even whole milk can be given

intravenously to calves without detrimental effect.

The purpose of our experiments carried out so far has been to investigate the ability of the animal to tolerate the intravenous administration of species-specific milk. The risk of sensitization was assessed by repeated injection of milk in the same animal. The animals were kept under clinical observation, and the temperature and respiratory and pulse rates were recorded. Other tests, including electrophoresis and determinations of blood cholesterol, blood sugar, and blood calcium were made.

### Material and Methods

All our experiments were performed on young calves, and ordinary cow's milk was used. The milk was usually injected into the jugular vein either rapidly or as a drip. The milk was collected under conditions as aseptic as possible, and in most cases was given quite untreated (in some experiments it was subjected to some form of pasteurization). Experiments have up to the present been carried out on 6 different calves which together have received 24 intravenous infusions of milk. The amount of milk infused on each occasion varied between 50 ml and 1 litre [*sic*]. A complete account is given below of two of the experiments in which the blood analyses mentioned above were carried out. Some data of the other experiments are given in Table 2.

#### Calf 1

This calf was given in all 4 intravenous infusions of milk. Cow's milk collected aseptically from an animal other than the mother was used. The milk was not treated in any way. The first experiment was carried out when the calf was two days old, when 60 ml of milk was injected rapidly into the jugular vein. No side reactions whatsoever were observed. The next experiment was made when the calf was four days old, when it was given 120 ml of milk in the same way, again quite

without side reactions. In the next experiment when the calf was 12 days old 220 ml was given. There were no side reactions. The last experiment on this calf was performed at the age of 32 days, 500 ml being given by intravenous infusion over a period of 21 minutes. In this experiment there was a slight increase in the respiratory rate, and during the following hour the calf was rather sluggish.

The animal continued to progress normally. A 20-ml blood sample was taken after every infusion. Electrophoresis revealed that despite a milk admixture of 10-15% in the blood, the only divergence from the normal electrophoretic pattern was a slight increase in the  $\gamma$ -globulin fraction. This must mean either that milk proteins disappear very quickly from the blood-stream, or else that an interaction takes place between serum proteins and milk proteins resulting in non-separable complexes. Certain preliminary experiments favour the latter alternative.

#### Calf 2

This calf was given the first injection at the age of 12 days. 500 ml was given in the course of 55 minutes. As before, the milk was collected under aseptic conditions, but was subsequently kept in a refrigerator for 24 hours. During the introduction of the milk the respiratory rate was slightly raised, and the animal was rather sluggish for the next hour or so after the completion of the experiment. Next day the calf appeared to be quite normal. During this experiment estimations were made of the blood sugar, total blood protein, blood cholesterol, and blood calcium. These figures are given in Table 1.

The second experiment on this calf was carried out when the animal was 16 days old. This time 450 ml of milk was given over a period of 65 minutes. On this occasion the side-reactions were more marked than in the earlier experiments. The respiratory rate was temporarily markedly increased, and the animal's general condition after the experiment was not quite normal. The total protein and blood sugar were again traced and showed changes largely similar to these in the previous experiment. The third experiment on

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TABLE 1.

Time, min. after commencement of injection	Milk injected ml	Blood sugar, mg per 100 ml	Protein %	Cholesterol, mg per 100 ml	Ca, mg per 100 ml
35	300	218	4.9	85	10.4
55	200	408	6.0	100	20.5
90		363	6.2	85	16.5
115		309	4.4	90	14.8
135		303	4.4	90	—
Control sample before injection	0	127	5.1	85	11.5

this calf was carried out to elucidate the effect of heating the milk before infusion. Aseptically drawn milk was therefore heated to 85°C for 5 minutes, a very drastic measure. This milk was injected by the same method as before, but when 150 ml had been administered the calf died. This experiment suggests that such rough treatment of the milk as was carried out results in changes which make it useless for intravenous nutrition. Subsequent experiments have shown that carefully performed heat treatment of the milk is associated with no side-effects.<sup>1</sup>

In Table 2 the results of some further experiments are summarized. Complete chemical data with regard to blood analyses are not yet to hand for these experiments.

TABLE 2.

Calf no.	Age in days	Amount of milk given ml	Side reactions
3	1	100	Tachypnoea
3	2	200	None
4	35	50	None
4	43	100	None
4	48	200	Slight sluggishness
4	52	300	Slight sluggishness and tachypnoea
4	57	300	Sluggishness, tachypnoea
4	61	300	None
6	2	800	None
6	3	1100	None
6	4	1000	None

In the first experiment, calf 3 received colostrum from its own dam. In all the other experiments mature milk from a cow other than the mother was given. In those cases where the temperature was measured in connection with the infusions a temporary rise of  $\frac{1}{2}$ –1°C for about 1 hour was noted.

### Discussion and Conclusions

As shown by the experiments it is possible, without producing serious side reactions, to give large volumes of whole milk intravenously to calves. Taking into consideration that the weight of the animals varied between 30 and 90 kg, the amount of milk administered was considerable, and in certain cases amounted to at least 25 % of the blood volume. It is of special interest that the natural fat emulsion of milk can be given intravenously without producing fat emboli. In the experiment in which the calf died, the fat emulsion had been changed by heating. In no case were there any signs of protein sensitization. This was indeed scarcely to be expected, as cow's milk was used. The experiments are being continued to elucidate to what extent the proteins of milk can be metabolized without first passing through the alimentary canal.

<sup>1</sup> Calf no. 5 was successfully given milk treated in different ways. All details of this experiment will be published later.

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THE PROCEEDINGS  
OF THE TWELFTH NORTHERN  
PEDIATRIC CONGRESS

HELSINKI  
JUNE 29—JULY 2  
1958

EDITED BY  
NIILO HALLMAN  
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YLPPÖ, LEA, Dr., Helsinki  
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ÖSTERLUND, KALLE, Dr., with wife, Helsinki.

## MINUTES OF THE PROCEEDINGS

*Monday, June 30th, 9 a.m.*

The President, Professor C.-E. RÄIHÄ, opened the Congress. He proposed as Vice-Presidents:

Dr. CARL FRIDERICHSEN, Copenhagen  
Prof. STURE SIWE, Lund  
Prof. LEIF SALOMONSEN, Oslo  
Prof. Bo VAHLQUIST, Uppsala  
Prof. TOIVO SALMI, Turku

### SESSION I

*Monday, June 30th, 10—12 a.m.*

Dr. C. FRIDERICHSEN took the chair.

Main Topic of the Discussion: Perinatal Mortality and its Prophylaxis.

Main Speakers: ARNE KÅSS, Norway, GUNNAR ENGLERSON, Sweden, ARVO YLPPÖ, Finland.

Other Speakers: AASE SKOGRAND, Norway: The Cause of Death in Premature Infants.

FRITZ FUCHS and GEORG STAKEMANN, Denmark: An Endeavour to Reduce Neonatal Mortality Through Treatment of Threatened Premature Labour with Large Doses of Progesterone.

ROLF LUNDSTRÖM, Sweden: German Measles during Pregnancy and Immaturity.

Discussion: E. K. AHVENAINEN, Finland  
R. RINVIK, Norway  
J. LIND, Sweden  
A. SUNDAL, Norway  
P. KARLBERG, Sweden  
C.-E. RÄIHÄ, Finland  
R. LUNDSTRÖM, Sweden  
J. STRÖM, Sweden.

## SESSION II

*Monday, June 30th, 2—5 p.m.*

Prof. S. SIWE took the chair.

Speakers: F. ADAMS, P. KARLBERG and J. LIND, Sweden: Circulatory Adaptation at Birth.

P. KARLBERG and G. KOCH, Sweden: The Lung Function during the First Minutes of Life.

S. JÄYKKÄ, TUOMAS PELTONEN and LEO HIRVONEN, Finland: Capillary Erection in the Living Lung.

SVEN SJÖSTEDT, GÖSTA ROTH and FRANCO CALIGARA, Sweden: The Oxygen Tension of the Amniotic Fluid during Administration to the Mother.

Discussion: C.-E. RÄIHÄ, Finland  
S. SJÖSTEDT, Sweden

GUNNAR CHRISTIANSSON, Sweden: Some Characteristics of the Blood Plasma Chemistry in Newborns.

Discussion: S. SJÖSTEDT, Sweden  
G. CHRISTIANSSON, Sweden

G. ENGLESON, G. ROTH and S. SJÖSTEDT, Sweden: Treatment of Premature Infants with 15% Oxygen.

Discussion: P. KARLBERG, Sweden  
C.-E. RÄIHÄ, Finland  
G. ENGLESON, Sweden.

F. BENSON and O. CELANDER, Sweden: Respirator Treatment of Pulmonary Insufficiency in the Newborn.

Discussion: S. JÄYKKÄ, Finland.

LARS ENGSTRÖM and BJÖRN IVEMARK, Sweden: Infection of the Fetus during Labor.

ÅKE LUNDBERG, Sweden: Paroxysmal Atrial Tachycardia and Atrial Flutter in the Neonatal Period.

ANDREAS KILLANDER and STIG SJÖLIN, Sweden: Indications for Exchange Transfusion in Icterus Neonatorum.

Discussion: P. PLUM, Denmark  
 G. CHRISTIANSSON, Sweden  
 M. SKATVEDT, Norway  
 P. W. BRÆSTRUP, Denmark  
 S. SJÖLIN, Sweden.

HOLGER DYGGVE and GEORG MUNK-ANDERSEN, Denmark:  
 A-B-O Sensitization in the Newborn, Diagnosis, Frequency  
 and Symptoms.

### SESSION III

*Tuesday, July 1st, 9—12 a.m.*

Prof. L. SALOMONSEN took the chair.

Main Topic of the Discussion:

The Etiological Background of Disturbances in Micturition.

Main Speakers: JÖRGEN VESTERDAL, Denmark, C. C. WINKEL SMITH, Denmark, ODD GARDGARD, Norway, BERTIL HALLGREN, Sweden.

Other Speakers: MARTIN SEIP and K. HARNAES, Norway: Arterial Hypertension in Unilateral Kidney Disease.

PER LUNDIN and INGEMAR OLOW, Sweden: Cystic Kidneys in Newborns, Infants and Children.

JAN WINBERG, Sweden: Renal Concentration Capacity during Acute, Nonobstructive Urinary Tract Infections in Infancy and Early Childhood.

K. V. PARKKULAINEN, Finland: On the Prognosis of Urinary Tract Infection in Childhood.

Discussion: P. W. BRÆSTRUP, Denmark.

KNUD WILKEN-JENSEN, Denmark: Nocturnal Bedwetting; An Attempt to Treat School Children with Banthine and Pro-Banthine.

Discussion: A. BIERING, Denmark  
 R. RINVIK, Norway  
 P. PLUM, Denmark  
 P. W. BRÆSTRUP, Denmark  
 B. HALLGREN, Sweden  
 P. PLUM, Denmark  
 J. WINBERG, Sweden

## SESSION IV

*Tuesday, July 1st, 2-5 p.m.*

Prof. B. VAHLQUIST took the chair.

Speakers: S. GARD, M. BÖTTIGER and R. LAGERCRANTZ, Sweden:  
A Field Study with Live Attenuated Polio-Vaccine.

Discussion: L. PHILIPSON, Sweden.

ROLF LUNDSTRÖM and ÅKE ESPMARK, Sweden: Immune  
Globulin Against Vaccinia.

Discussion: T. SALMI, Finland.

LENNART PHILIPSON, Sweden: Virologic Aspects on the  
Upper Respiratory Infections in Childhood with Special  
Reference to Non-Diphtheritic Croup.

Discussion: R. LUNDSTRÖM, Sweden  
R. LAGERCRANTZ, Sweden  
L. PHILIPSON, Sweden.

O. BROBERGER, I. JUNGNER and R. ZETTERSTRÖM, Sweden:  
A Rediscovered Etiological Factor in Idiopathic Hypo-  
glycaemia in Children.

Discussion: R. RINVIK, Norway  
Z. ERIKSSON-LIHR, Finland.

TOIVO SALMI, AIMO PEKKARINEN and SAARA HEIKKILÄ,  
Finland: The Adrenocortical Function in Children.

STEN WIDELL, Sweden: Studies on the Cerebrospinal Fluid  
Protein in Childhood.

MARIT SKATVEDT, SIGURD EEK and ODD GARBORG, Norway:  
Epilepsy in Children. A Clinical and Roentgenological Study.

Discussion: P. PLUM, Denmark  
S. EEK, Norway  
M. SKATVEDT, Norway

CLAES THORÉN, Sweden: Cardiopathy in Friedreich's Ataxia.

## SESSION V

*Wednesday, July 2nd, 2--4 p.m.*

Prof. T. SALMI took the chair.

Main Topic of the Discussion: The Somatic Development of the Child and Factors Influencing It.

Main Speakers: O. MELLANDER and B. VAHLQUIST, Sweden, E. THAMDRUP, Denmark, HOLGER HULTIN, Finland.

Other Speakers: M. SEIP, Norway: Iron Requirements in Infancy.

Discussion: K. WILKEN-JENSEN, Denmark

B. VAHLQUIST, Sweden

K. KALJSER, Sweden

H. LICHTENSTEIN, Sweden

B. VAHLQUIST, Sweden

C. FRIDERICHSEN, Denmark



## PRESIDENTIAL ADDRESS

C.-E. RÄIHÄ

Honoured representatives of the State, Helsinki University, the Town of Helsinki, honoured invited guests, Honorary Members of the Nordic Pediatric Association, ladies and gentlemen, colleagues and friends . . .

It is with great pleasure that I bid you welcome to the XIIth Nordic Pediatric Congress. This greeting is extended to you, Scandinavian pediatricians with your wives and families, not only from the warm hearts of Finnish colleagues, it is extended to you from the whole Finnish nation. The year which has gone has left memories which bring the children of the North closer to each other, and the pediatricians of the North have had and have as a consequence an important mission to fulfil. Our work and our activity are reflected in the growing health and fitness of the race. The community needs, to a continuously increased degree, advice in technical knowledge, and thus these our regular Nordic congresses are important. Our Nordic communities are built upon a common culture, our geographical situation characterizes our problem, for centuries bonds of friendship and kinship have united our peoples. This is reason enough for us to meet, discuss our problems, get to know one another, revive memories, formulate plans for future cooperation, and pass on to coming generations what we have obtained from the generations who have left our circle.

Since we last met in Oslo, the following colleagues have passed away: Professor YNGVE ÅKERRÉN, the First Professor in Pediatrics in Gothenburg: a good teacher and doctor, an enthusiastic research worker, who with experience and knowledge took a personal standpoint in discussions on experimental and clinical results. He was a good administrator and hospital director. Within the Nordic Pediatric Association, his friendly nature and his contribution to prophylactic care of children during the new-born period will not be forgotten for a long time to come.

Dr. SVEN RUDBERG, practising pediatrician in Stockholm, was well-liked, put the needs of others before personal considerations, and was a good colleague.

Professor ARMAS RUOTSALAINEN, one of Finland's first pediatricians, was a man who had been the teacher of our oldest surviving Finnish colleagues at the Children's Clinic in Helsinki. We remember his distinguished and considerate nature, and his special interest in the people's medical methods of earlier days.

Docent KARL RAFAEL KYRKI, one of the first and best known pediatricians of Turku, with a special interest in children's tuberculosis.

Doctor USKO IMMANUEL MUROMA, a highly appreciated pediatrician in Helsinki, where he worked with the Child Welfare Committee and as a school doctor.

Let us with a moment's silence honour those who have passed on.

The moment we call «now» is short, and our thoughts speed from memories of what has been, and from the strivings of past generations, to duties towards the generations to come, and the formulation of future plans.

The Nordic Congress in Pediatrics, which I have today the honour of opening, is the twelfth in order of the congresses which have been held since 1919, that is to say during nearly forty years. The first Pediatric Congress in the North was convened in Denmark, where the thought of Nordic collaboration originated five hundred years ago. At the venerable University of Uppsala, in the centre of the Nordic cultural circle, scientific pediatrics was born two centuries ago. This gives us Nordic pediatricians the duty of common continued efforts.

Before the first world war, there arose the thought, first of all on the part of Carl Looft in Bergen, that Nordic pediatrics should be developed by means of combined congresses, and through a mutual Nordic Pediatric Association. In order to get a clearer picture of the early history of our meetings, I wrote this spring to Professor WILHELM WERNSTEDT, now 85 years of age. His reply was very comprehensive, and from it I quote the following:

»Fortunately, I can provide an answer to your question, as I was one of those — indeed the only one now living — who was involved from the beginning.

The actual one to give the initiative was CARL LOOFT in Bergen. Almost 45 years ago to the day — the 4th March 1913 — I (WERNSTEDT) had a letter from him, in which, amongst other things he said that at the Nordic Congress for Internal Medicine in Bergen, in 1911, he had spoken with BLOCH of the possibilities of building a Nordic pediatric association in connection with these congresses».

And further . . . . »In 1913, accordingly, in Lund, there was a meeting of LOOFT, FRÖLICH, MONRAD and the undersigned (WERNSTEDT). We were all in agreement that something should now be done to break us pediatrici free from the authority of internal medicine. And then came the war.»

And, in addition . . »It is possible that my memory is at fault in one or two details of what I have quoted above. But so much is absolutely certain, that LOOFT was the actual originator in the building of the Nordic Pediatric Association, and that he, FRÖLICH, MONRAD, ADOLF MEYER, LÖVEGREN and the undersigned (WERNSTEDT) constituted the first association to accomplish the object.»

With this, the beginning was made, and I have in brief wanted to touch upon the early history of our congresses, as soon it will be a half century since the initiative was taken.

The war finished in 1918 swept away a period of time which had begun with the French Revolution, and which was characterized by national strivings. In pediatrics, this time was the beginning of what we now call the social welfare of children and clinical pediatrics. The overshadowing problems were then connected with nutrition and infectious diseases, which occasioned mass mortality and which prevented growth of the population despite a very high birthrate. Since then, the picture has changed completely. Infectious diseases and starvation as causes of death have amongst us practically disappeared, since it has been shown that in large population groups this problem can be solved through a raised standard of living and education. The field of activity in our special branch, pediatrics, has simultaneously been broadened. We must nowadays establish contacts, which are essential for the development of pediatrics, with all medical specialities, sciences, and with social research, to a continuously expanding extent. The pediatrician should cooperate with the theoretical research worker, but at the same time participate in the practical development of the community. At this Congress, there will be discussed some of the problems which confront us. Perinatal mortality, a problem which cannot in any way be attacked without increased cooperation with the obstetrician — the problem of growth, which demands augmented contacts with sociology, genetic and theoretical sciences — the significance of the anatomic structure in functional disturbances will be discussed at this Congress in connection with urination troubles. This last named illness demands intensified cooperation with surgery, in which a new, special domain, that of infant surgery, is being developed. Pediatrics will become to a greater and greater extent a cooperation between highly specialised experts. The question of increased contacts between specialists in internal medicine is becoming increasingly topical, as many of the problems we face, since pediatrics has grown to its present form, are identical. Already, there is the University, in which medical problems relating to the age of childhood are dealt with in a special faculty.

The problem which confronts us increases in importance with our increased knowledge, it demands increased contacts with other cultural circles as well as an increased and more intensive cooperation between Nordic colleagues. I hope that everybody will get along well in this way, and will feel the time spent together as of importance for coming tasks: and I wish that the personal contact will forge links between Nordic colleagues which will bring us all nearer one another. I have the honour to declare the 12th Nordic Pediatric Congress open.

Before we leave this hall and begin our work, I also have the honour to convey to the Town of Helsinki our warmest thanks for the reception we have had.

I hope that the Congress will give its approval to the following proposals of the Board:

1) That the Congress sends a telegram of greetings to Mrs. ALLI PAASIKIVI, widow of our late President. Mrs Paasikivi has continuously shown the greatest interest in our work.

2) That the Congress sends a telegram of greetings to Mrs KARIN ÅKERBÉN.

3) With us, we have our highly respected Honorary Members Dr CARL FRIDERISCHEN and Archiater ARVO YLPPÖ. The Board suggests that we send telegrams of greetings to the absent Professor AF KLERCKER, Lund, Professor THORLING, Uppsala, Professor WERNSTEDT and Professor WALLGREN, Stockholm, as well as to Dr ARTHUR COLLETT, Norway.

The Board proposes the following five Vice-Presidents for this Congress: Dr CARL FRIDERICHSEN, Professor STURE SIWE, Professor LEIF SALOMONSEN, Professor BO VAHLQUIST and Professor TOIVO SALMI.

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## SESSION I

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## Perinatal Mortality and its Prophylaxis, Prenatal Factors

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The infant mortality rate has shown a very rapid decrease in this century, mainly due to different preventive and therapeutic measures. It is especially the later mortality of infants, that is to say death between 2—52 weeks of life which has been reduced, whereas it is the early neonatal mortality, namely death in the first week of life, which has decreased to a much lesser extent.

Sweden has the lowest infant mortality in Scandinavia, and also in international statistics (1956: 1.73%), but when one considers early neonatal mortality Norway is the best as far as international statistics are concerned (1956: 1.05%).

The early neonatal and the late foetal mortality are together known as the perinatal mortality. Calculated per 1,000 living births, this rate is about 0.3% in countries with a high standard of living.

Since perinatal deaths form a greater proportion of the overall infant mortality, there is an increased demand for experimental and clinical investigations with the aim of reducing this fraction. Though we cannot draw a sharp aetiological distinction between the two forms, we must continue to strive towards the greatest possible reduction of these deaths. The aetiology of perinatal death, according to the material under consideration, seems to consist of the following factors, which often overlap.

### *1. Premature births.*

This condition assumes a very important place in infant mortality, but will never give an adequate explanation for intrauterine death, and only rarely the complete explanation for neonatal death. Even though exact clinical diagnosis and post mortem examinations reduce the frequency of prematurity per se as a cause of neonatal death, prematurity as a dominating and contributory factor will render more difficult the prognosis of any other pathology.

### *2. Malformations of the child.*

Embryopathy is more often than not of unknown aetiology, and is therefore outside the sphere of possible prophylaxis. Newly recognized factors in the first trimester causing malformations of the child are as follows:

- Viral infections (small pox vaccination)
- Irradiation injury (mutations)
- Cortisone treatment

Anoxic conditions (changes in foetal milieu, gynecological diseases.)

Probable causative factors: Nutritional disorders. Acute or chronic conditions of «stress».

### 3. Placental conditions.

Dysfunctions — nutritional disturbances, pre-eclampsia, senile regressive changes, abnormalities of the cord — placental insufficiency: Foetal asphyxia, pseudo-prematurity, the postmaturity syndrome, foetal maceration.

Abnormal placental position — maternal haemorrhage, foetal asphyxia.

Abnormalities, «scars» etc. — foetal haemorrhage, foetal anaemia and malnutrition.

### 4. Birth trauma.

Dystocia, prolonged labour, drugs to the mother, hypo-oxygenation of the mother — intrauterine foetal asphyxia.

Trauma during labour, precipitated delivery, artificial delivery — intracranial haemorrhage.

Foetal malpresentation. Breech. Transverse lie.

### 5. Intrauterine infections.

Lues.

Protozoal infections (toxoplasmosis).

Viral infections (congenital hepatitis etc.)

Bacterial infections (pneumonia).

### 6. Iso-immunization of the mother.

Haemolytic disease of the newborn.

### 7. Diseases of the mother.

Diabetes of the mother — increased frequency of defects and infant mortality.

The greater the degree of prematurity and underdevelopment of the child, the greater the contribution will be to the other causative factors mentioned above. Premature babies dominate the perinatal statistics, and make up about fifty per cent of still-births and neonatal deaths.

My own observations from Kvinneklippen, Bergen, from the years 1941–50 include 25,500 births, the frequency of prematurity being 4.9%. 41% of all still-born babies had a birth weight of less than 2,500 g, and 52% of babies who died in the first 7 days of life were also premature. 1,246 children with a birth weight of less than 2,500 g had a still-birth rate of 15.7%, an early neonatal mortality rate of 15.3%, and altogether a perinatal mortality rate as high as 31%.

Analysis of the available data concerning the biological, health and social

conditions of the mother showed the same relationship between the frequency of prematurity and the perinatal mortality of the children.

Mothers between 25 and 29 years of age had the lowest number of premature births, and also had the lowest perinatal mortality rate of the babies. The increase in the perinatal mortality rate for the youngest mothers is due in particular to the increase in the early neonatal mortality, while for the older mothers it is due to an increasing still-birth frequency.

Uniparous mothers have the fewest premature infants, and also the lowest perinatal mortality of the infants.

In 51% of cases, mothers giving birth to premature infants had symptoms of pre-eclamptic toxæmia. It seemed that as the age of the mothers with pre-eclampsia increased, so did the number of premature infants. For mothers with premature babies, the incidence of pre-eclampsia increased from 40% to 70% with the increasing age of the mother. Proteinuria and hypertension which we diagnosed in pregnant women and women in labour resulted in 18% of the babies being born prematurely, and about 8% perinatal mortality. 17,300 mothers without symptoms of pre-eclampsia during pregnancy or while in labour gave birth to infants of whom 3.5% were premature, and only 2.5% died perinatally.

Mothers from the lowest social classes (IV & V) had a higher frequency of premature infants (8%), and also the highest perinatal mortality rate of the infants. The same relationship was also found for unmarried mothers.

The mothers who had regular antenatal care by their doctors had a lesser degree of prematurity and infant mortality than did the mothers who had failed to attend or who had attended only irregularly.

Diseases of the mother, haemorrhage during the pregnancy and malformation of the child seem to have had a great importance in individual cases, but are only of minor importance from the point of view of total mortality.

One is left with 14,100 deliveries in specially selected cases which do not include unmarried mothers, those with disease, haemorrhage or signs of pre-eclamptic toxæmia. Once again, one sees in this group the lowest prematurity rate amongst mothers between 25—29 years of age, and uniparous mothers. Further analysis of the material shows that it is the age rather than the number of pregnancies which is of biological importance.

It is also clearly seen from the figures that abnormal obstetrical circumstances such as breech deliveries, twins, precipitated labour and prolonged labour cause a definite increase in the mortality rate of the infants.

In order to diminish the perinatal mortality rate we must direct our prophylactic measures especially towards prenatal and natal causative factors which are already known. We must first of all try to prevent premature births. We consider that we have an effective method in that we can achieve proper antena-

tal care, which, in addition to the ordinary medical control, also puts the emphasis upon optimal nutrition and hygiene for the mother. The aim must be that this control and the possibilities for further care are given in particular to those having the highest incidence of premature babies i.e. the unmarried mothers, nulliparous and mothers of the lower social classes. One must take every precaution against factors endangering the foetus (malnutrition, viral infection, irradiation and «stress» situations of the mother) especially in the first trimester. The danger of congenital syphilis and the risk for the child with erythroblastosis can be reduced to a minimum by blood investigations of the mothers and further necessary means. Obstetrical help has to be of an expert nature in order to eliminate the risk of birth trauma and asphyxia neonatorum, often brought about by poor assistance being given to the mother, or uncritical medication being provided.

If our prophylactic measures are going to become better and more efficient, it is necessary that the causative factors must be found to as great an extent as is possible. In order to increase our knowledge of prenatal causative factors of perinatal mortality, it is necessary to take exact histories from the mothers of the dead infants. One must further seek to determine the exact cause of death in still-birth and early neonatal deaths, and it is also important to aim at reliable and uniform statistics and material for further scientific work.

The accomplishment of this important medical task is of a hygienic and obstetric nature; the pediatrician has also an important part to play in that he endeavours to find the causative factors and treat the newly born infants adequately in order to reduce the effects of the prenatal injuries.

## **Perinatal Mortality and its Prophylaxis: Postnatal Factors**

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The concept «perinatal mortality» has been used increasingly during the last few years. It refers to the number of stillborn and neonatal deaths per 1,000 births (i.e. both liveborn and stillborn children).

The increase in the average length of life observed in most countries can be ascribed, as is well known, to a considerable extent to the appreciable decrease in infant mortality. Low infant mortality is especially noteworthy in the Nordic countries. It has been found, however, that the drop in infant mortality coincides predominantly with a certain period in the first year of life, viz. from the 2nd month of life, i.e. after the end of the neonatal period. Mortality

within the neonatal period and especially within what is called the early neonatal period, corresponding approximately to the first week of life, is still relatively high. At all events, the decrease in mortality is considerably smaller in this early period.

The aim of introducing the concept perinatal mortality has been among other things the intention to analyse the different factors that influence this still relatively high early mortality.

As regards prophylactic measures against perinatal mortality, it is obvious that the role of the pediatrician is confined essentially to liveborn children. Prophylactic measures for stillborn babies, the frequency of which is roughly comparable to the frequency of neonatal deaths, concern primarily the obstetrician.

In discussing the role of postnatal factors in perinatal mortality we can more or less strictly define what is implied by postnatal factors. Strictly taken postnatal reasons can be limited to cover a condition of purely postnatal origin. Accepting this, the question is almost exclusively one of infections of postnatal occurrence, hemorrhagic disease of the newborn, atelectases, hyaline membranes, etc. As far as incidence is concerned, conditions of this type account for only some 15 per cent of total perinatal mortality and thus comprise no dominant factor. A pediatrician may, however, be considered entitled to extend the concept of postnatal factors, especially since both prophylactically and therapeutically the role of the pediatrician covers a number of other conditions which occur after the birth of the baby irrespective of whether they are primarily related to other factors affecting perinatal mortality.

Only a number of preliminary facts can be mentioned in the present survey. Certain complications which occur during the mother's pregnancy and during parturition itself are known of old as possible causes of the death of the baby during the neonatal period. Suffice it to mention here factors such as toxemia of pregnancy, Rh-incompatibility, Cesarean section, forceps delivery and labour protracted in some way or another. In large series these factors account for around 20—22 per cent of perinatal mortality.

Prematurity per se naturally constitutes an important factor for perinatal mortality, but it is often also a cause, together with other factors, e.g. toxemia, Rh-immunisation, contributing to reduce the newborn baby's chances of survival.

Postmaturity and prolonged pregnancy are often confused, and the role of these conditions in perinatal mortality is at present attracting great interest.

To avoid misunderstandings, it should be emphasized that prolonged pregnancy means only pregnancy exceeding the normal duration, while postmaturity (dysmaturity) or placental dysfunction is a special condition in the newborn. CLIFFORD, who has studied these conditions, is of the opinion that postmaturity

plays as great a role as prematurity in perinatal mortality. The speaker reported his own investigations in this field.

Congenital malformations, which cannot be considered primarily to belong to the postnatal factors, account for a certain percentage, c. 7—8, of the total perinatal mortality.

Only a few data will be mentioned here regarding prophylactic measures, i.e. the possibilities of reducing perinatal mortality, with special attention to the role of the pediatrician.

As stated above, the pediatrician enters the scene principally during the period after the baby's birth. Many pediatricians are familiar with the measures taken during this time. They may be regarded as consisting chiefly of early examinations upon the birth of the child, prophylaxis against infections, K vitamin therapy, care of premature and postmature children and similar measures. Every maternity ward must have access to immediate pediatric expert knowledge. This applies specifically to complicated deliveries of various types, Rh-immunisations, maternal diabetes, etc., viz. conditions which are known to play a major role in perinatal mortality.

The pediatrician must also be able to advise the obstetrician in different conditions complicating pregnancy.

The lecture was illustrated by statistical data on the share of the different factors in perinatal mortality.

## Perinatal Mortality and its Prophylaxis

ARVO YLPPÖ

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The *marriage advice centre* can be regarded as the first stage in our endeavours to reduce perinatal mortality. The work must be focussed primarily on hereditary-hygienic problems and not merely on birth control.

*Maternity counselling*, if it covers all classes of society and the pregnancy as a whole, is of great importance. This is evident in our country where the Act on Maternity Centres took effect in 1944. Congenital lues has disappeared almost completely, the number of premature babies has dropped from eight to five per cent of all births and to 4 per cent of live births. The number of stillbirths has diminished during the same period from 2.2 to 1.8 per cent.

There is a direct relationship between complications during delivery and antenatal mortality. Various intrauterine infections, both of bacterial and

viral origin, contribute more often than is realised to antenatal mortality. Congenital pneumonia, for instance, is no rare condition.

*Expert care during the very first minutes* after delivery may save a baby's life. This applies first and foremost to the treatment of babies in a state of suspended animation accompanied by respiratory disturbances. Special demands are made *not only* on the physician, the obstetrician, but also on the *entire assisting staff*.

For instance, the greater the knowledge of *midwives* about children and the care of children — a special course in children's nursing is desirable — the better will she be able to help and nurse the child throughout the perinatal period.

### The Cause of Death in Premature Infants

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As was mentioned in the introductory lectures, premature infants dominate in the perinatal mortality statistics. Until adequate methods to prevent premature births have been found, attention must be directed toward the factors, in addition to the prematurity itself, that are responsible for the high mortality rate. On careful autopsy of the prematures it appears that pathological lesions which can explain the occurrence of death are present in the great majority of the cases. Only a very few remain for whom the prematurity itself must be accepted as the sole cause of death, and especially in the weight-group under 1,000 gms.

A study of the premature material from the Pediatric Clinic in Oslo for the four year period 1954—1957 showed the following distribution among the various weight-groups: <1,000 gms: 18 cases, 1001—1500 gms: 113 cases, 1,501—2,000 gms: 162 cases, 2,001—2,500 gms: 63 cases, i.e. a total of 356 cases. The mortality rate was 34%. Within the various weight-groups the mortality was: 78%, 50%, 20% respectively, and 25% in the heaviest weightgroup. These values are not directly comparable with the mortality percentage reported by other pediatric clinics. A great variation in the mortality rate in the different statistics is ascribable to several factors. The statistics have been compiled at different times, the age of the infants on admittance to the clinic may vary, some having been born at home or in out-of-town hospitals, and another important factor is the distribution among the various weight-groups. The present series shows a remarkably high mortality rate for the two heaviest weight-groups. This is

due to the fact that of the larger prematures, only those with some ailment or other are admitted to the Pediatric Department. Of the premature babies born in the Obstetrical Department of Rikshospitalet, 100% in the weight-group under 1,500 gms are transferred to the Pediatric department, 60% in the weight-group 1,501—2,000 gms, and only 30% in the weight-group 2,001—2,500 gms.

The pathologic-anatomical findings from the autopsies were the following: intracranial hemorrhage: 22 cases, pulmonary atelectasis: 22 cases, pulmonary atelectasis with hyaline membranes: 43 cases, pneumonia: 14 cases, pulmonary hemorrhage: 9 cases, kernicterus: 5 cases, malformations: 2 cases and miscellaneous: 3 cases.

It should be emphasized that the individual cases usually present various pathological lesions, and determination of the direct cause of death must to some extent depend on an estimate. The same approximate estimate of the findings will evidently occur also in the series from other clinics, which adds to the uncertainty in the comparison of the different statistics.

Further, it should be stressed that pulmonary atelectasis is a purely descriptive term, not to be conceived as a specific cause of death. But as this is the only pathological finding in these autopsies, it has been considered expedient that they should be collected into one group. Nine of the cases belong to the weight-group under 1,000 gms, and thus the immaturity itself is the direct cause of death. The diagnosis of atelectasis in the heavier weight-groups merely conceals the fact that the direct cause of death is unknown. In some of these cases, the death is probably due to intrauterine asphyxia and consequent injury to the respiratory centre.

Of the 22 cases of intracranial hemorrhage, the bleeding was intraventricular in 14 and subdural in 8. Moderate subarachnoidal bleedings were common autopsy findings, but these were taken as secondary to anoxia.

Pulmonary atelectasis with hyaline membranes represents the largest group, and the majority of these cases died in the course of the first 2 days of life (see the table). Massive intraalveolar hemorrhage as the cause of death occurs 2—4 days and kernicterus 7—10 days postnatally (see the table). Of malformations there are 2 cases, one of esophagus atresia and one of congenital heart defect. — The group of miscellaneous includes one case of volvulus of the small intestine, another, intestinal perforation with peritonitis, and one of fetal hydrops.

According to the table, most deaths from pneumonia occur in the course of the first 3 days of life, which indicates intrauterine infection. The aspiration of major quantities of amniotic fluid occurs — as known — on intrauterine asphyxia. It thus seems reasonable to presume that asphyctic brain injury is a contributory cause to the poor prognosis for aspiration pneumonias.

No considerable reduction of the mortality rate of the prematures can be expected before more is known about the factors — besides immaturity — that lead to the occurrence of hyaline membranes in the lungs and to pulmonary and intracranial hemorrhages. Detailed autopsy material will therefore be of great importance to continued study in these fields.

TABLE 1

	1. day	2. day	3. day	4—7 day	2. week	3. week	4. week	after 4. week
Intracranial hemorrhage ....	9	6	3	4				
Atelectasis .....	8	4	3	5				2
Atelectasis + hyaline membr. ....	20	19	3	1				
Pneumonia .....	4	3	4	1		1	1	
Pulmonary hemorrhage ....		4		5				
Kernicterus .....				3	2			
Congenital malformations ..			1		1			
Miscellaneous .....	1			2				

### An Endeavour to Reduce Neonatal Mortality through Treatment of Threatened Premature Labour with Large Doses of Progesterone

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The dominant cause of death in the neonatal period is prematurity. Since the medical, social, and nutritional factors known to contribute to premature deliveries are difficult to evaluate in individual cases and therefore difficult to prevent, it would be of immense value to have a substance capable of inhibiting uterine contractions. In spite of the evidence of the role of progesterone in the hormonal control of pregnancy provided by animal experiments, opinions are divided with regard to the effect of progesterone in threatened abortion and premature labour. A double-blind controlled study of the effect of large doses of progesterone in patients with threatened premature labour has therefore been carried out.

The series included 126 patients, half of whom were treated with progesterone in oil intramuscularly in doses of 200 mg daily for 3 days, 150 mg for 2 days, and 100 mg as the maintenance dose. The other half of the patients were given similar doses of the same oil without progesterone, and not until after the

analysis of the series was it disclosed which of the two preparations, labelled A and B, contained progesterone. If the symptoms subsided, treatment was discontinued after a certain period of time.

The two groups were in close agreement with regard to age distribution, previous obstetric history, and symptoms. The results are shown in Table 1. It is seen that there is no difference between the two groups. Almost equal numbers delivered despite treatment, and those for whom delivery was successfully postponed were also equal in numbers. Group B received progesterone, Group A inactive oil.

TABLE 1  
DELIVERY IN RELATION TO TREATMENT IN THE TWO GROUPS, DIVIDED AFTER THE DOMINANT INITIAL SYMPTOMS

	Hæmorrhage		Passage of amniotic fluid		Rhythmic or constant pains	
	A	B	A	B	A	B
<i>Delivery during treatment</i>						
First or second day.....	4	4	6	7	3	2
3rd—7th day.....	2	0	5	1	0	1
8th—14th day.....	2	4	2	6	0	0
15th—28th day.....	0	1	2	2	0	1
After 28th day.....	1	1	0	1	0	0
	9	10	15	17	3	4
<i>Delivery after treatment</i>						
During first week.....	2	3	0	1	1	0
During second week.....	2	2	0	0	1	0
Third or fourth week.....	3	1	0	0	4	3
Later than four weeks.....	12	7	4	3	7	12
	19	13	4	4	13	15

It is concluded that progesterone is not the sorely needed substance which is able to inhibit uterine contractions in threatened premature labour and thereby reduce the neonatal mortality. It is imperative that the search for such a substance is continued and intensified.

### German Measles during Pregnancy and Immaturity

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In connection with a widespread epidemic of German measles in Sweden in 1951, a prospective study was undertaken to obtain an idea of the incidence of damage to the foetus of the type that is caused by German measles in the

mother during pregnancy. By questioning c. 100,000 women admitted to maternity hospitals during the period July 1, 1951—June 30, 1952, data were obtained on c. 1,000 children whose mothers had suffered from German measles during pregnancy.

A study of the birth weight of the children showed that the incidence of immature babies (birth weight = 2,500 g) was significantly higher in the section of the material with a history of German measles during the first part of pregnancy compared with the section showing the disease later during pregnancy and a control material selected at random. Congenital developmental disturbances, localised principally in the eyes, the auditory organs and the heart showed a higher incidence among the immature than among the babies with a higher birth weight.

A comparison was made between the incidence of developmental disturbances in the measles material and the control material in relation to immaturity.

An interesting point in this connection is C. Gyllenswärd's study of the incidence of immaturity in different maternal groups. Gyllenswärd found a relatively high incidence of low birth weights in children of young mothers who had moved to large towns from rural districts. As these mothers could be expected to be more liable to fall ill from contagious complaints than mothers who had grown up in a large town and had e.g. infectious diseases of childhood earlier, it is conceivable that the explanation for the higher incidence of immaturity among the children of the rural young women can be ascribed to a higher incidence of infection in this material resulting from the greater risk of infection present in the towns.

The present observation of immaturity as a sequela of German measles during pregnancy is probably not unique for this disease. It could very likely be established for other maternal viral diseases provided that a sufficiently large material were available for study.

#### DISCUSSION

*E. K. Ahvenainen, Finland.* — Data from an autopsy material of 1,247 newborn infants was presented. (Table 1). Traumatic intracranial hemorrhages include subdural hematoma, rupture of the tentorium with hemorrhage and fracture of the skull. «Other» includes intraventricular hemorrhage, softening of the brain and leptomeningeal (subarachnoid) hemorrhage. The incidence of intracranial hemorrhages varies in different statistics. This is partly caused by variable methods of classification. If leptomeningeal hemorrhages are included in the group of anoxia, as is the case in some statistics in the USA, the incidence of intracranial hemorrhages is lower than in this table. Other hemorrhages

TABLE 1  
DISTRIBUTION OF ANATOMIC CAUSES OF NEONATAL DEATHS, HELSINKI, CHILDREN'S CLINIC  
1947—1956

	Per cent of total	
	Premature	Fullterm
Intracranial hemorrhage		
Traumatic .....	6	8
Other .....	20	5
Other Hemorrhages .....	4	4
Malformations .....	14	40
Infections .....	20	19
Hyalinen Membrane.....	14	5
Kernicterus .....	10	8
Erythroblastosis without Kernicterus .....	1	4
Anoxia .....	2	2
Prematurity .....	5	—
Miscellaneous .....	4	5

are severe pulmonary hemorrhages, adrenal hemorrhages and hemoperitoneum due to rupture of the liver. These hemorrhages may according to the personal feeling of the pathologist be considered traumatic or as caused by anoxia.

Congenital malformations are important constituting the largest group. The incidence of congenital malformations is greater in this material than what can be supposed as being present in the whole of Finland, since such cases are concentrated in a teaching hospital with an active surgical ward.

Infections are more common than are to be seen in the table, since many newborn infants with congenital malformations or intracranial bleedings succumb on a secondary infection. In the group of pulmonary hyaline membranes, only such cases are included as those in which the membranes were the only autopsy finding.

In many cases Kernicterus was probably caused by Vitamin K. The incidence of this disease has decreased in recent years with the use of smaller doses of Vitamin K.

The anoxia group is smaller than it is in much other material. Only such cases are included as those in which clinical facts and autopsy finding made anoxia very probable, and no anatomic changes were seen which would have made it possible to include the case with some other group. The incidence of anoxia in different statistics varies according to those cases which are included. When the newborn dies shortly after birth, anoxia is often without doubt an important one of the many factors leading to death. That is why there are so many differences based on subjective opinion.

Abnormal pulmonary ventilation has been much employed as a diagnosis for

premature infants. In this group have been included cases with and without hyaline membrane, since Potter is of the opinion that the immaturity of the lungs is the most important factor in the death of an immature infant. Any diagnosis can be used when it is explained clearly, but one wonders whether it can be considered a proven fact that there is no difference between premature infants with and without hyaline membranes. I feel that things are made clearer when a differentiation is made between premature infants with and without hyaline membranes. For this reason, the prematures without other findings are presented in their own group.

TABLE 2

PERCENTAGE DISTRIBUTION OF ANATOMIC CAUSES OF NEONATAL DEATHS IN DIFFERENT PERIODS

	1947—49	1950—52	1953—56
Intracranial hemorrhage			
Traumatic .....	8	10	4
Other .....	7	13	20
Other Hemorrhages .....	4	5	3
Malformations .....	21	26	31
Infections .....	37	14	8
Hyaline membrane .....	4	14	13
Kernicterus .....	9	7	11
Erythroblastosis without Kernicterus .....	2	2	4
Anoxia .....	1	2	3
Prematurity .....	3	3	1
Miscellaneous .....	6	5	3

Table 2 shows the variation in different years. It is to be mentioned that infections are still an important factor as a secondary cause of death, in spite of their diminished incidence. The number of congenital malformations has not increased so much that it could be postulated that this is anything else but relative.

*R. Rinvik, Norway.* — Rinvik emphasises the importance of postnatal prophylaxis against infections in combating perinatal mortality. He reports on a material in which pyoderma was established in over 35 per cent of the newborn in a maternity ward where 3 nurses were staphylococcus carriers. When the nurses were removed the incidence fell to 11 per cent. 23.3 per cent of the mothers had mastitis during the first period (i.e. when 3 carrier nurses were present); the incidence dropped to 5.6 per cent during the second period. During the first period the incidence of symptom-free mothers and children was 48 per cent, in the latter period 80 per cent of them showed no signs of infection.

Antibiotics have given us a false sense of security. Infection by the hospital staphylococci is an indication of this.

Greater emphasis than before must be laid on prophylaxis against infections. How this should be performed is a debatable question, either through air disinfection of the rooms in one form or another, or through regular, frequently repeated controls of personnel who may be bacillus carriers. We may have to become so «radical» as to recommend that the delivery take place at home instead of in hospital.

*J. Lind, Sweden. —*

*A. Sundal, Norway. —* International comparative statistics on stillborn children and deaths in the first week, i.e. on perinatal mortality, depend firstly on the legislation prevailing and secondly on the accuracy and reliability with which the person assisting at the birth reports the occurrence.

Norwegian law on the subject is based on an Order in Council of 9th November, 1901 which states: «Births in which the embryo is born before the end of the 4th calendar month of pregnancy are not to be reported.» All births in which the embryo is born after the 4th calendar month should be reported to the Medical Officer of Health. The limit of 4 calendar months has been taken to mean 16 weeks of pregnancy but the duty of midwives and birth assistants to report to the Medical Officer of Health is not the same as registration to the Registrar of Births, Deaths and Marriages. Here the following regulation applies: «As liveborn are considered all children showing signs of life after birth, whether or not they die immediately afterwards. As stillborn are considered children born without life after the 28th week of pregnancy. Children born without life before the end of the 28th week of pregnancy need not be reported on the register.»

The regulations are thus clear: All conception products expelled from a woman after the 16th week of pregnancy are to be reported — liveborn as well as stillborn. But unfortunately some doctors and midwives erroneously do not report exactly: a child born with signs of life for a very short time may be reported as a stillbirth. And if very much premature it may also be reported — erroneously — as an embryo born before the 16th week of pregnancy. If we could obtain exact reports of the stillborn group, including birthweight, our perinatal mortality figures would be more uniform, as even the smallest liveborn children (500–1,000 g in weight) which die immediately after birth would at least appear in the statistics of stillborn children, even if they were erroneously registered.

Dr. Kåss's introductory lecture fully demonstrated the significance of prematurity in perinatal mortality. According to his investigations on a selected material from the Maternity Hospital, Bergen, prematurely born children had thirteen times as many stillborn and 26 times as many deaths in the first week as

children with a weight at birth of over 2500 g. In a completely representative material from a district in Norway with a particularly low infant mortality (Bergen 1955) and in which *all* births were registered, prematurely born children showed even more unfavourable figures: 19 times as many stillbirths and 58 times as many deaths in the first week, i.e. a perinatal mortality 28 times as large as that among full-term births.

*P. Karlberg, Sweden.* — A few years ago, I made a summary of the available official reports on the causes of perinatal mortality in Sweden since the introduction into the country of WHO's International Classification (see Figs. 1 and 2).

As is shown in Fig. 1, the diagnosis is very uncertain in over one half of the still-borns. Fig. 2 shows even less of a certainty for the cases of neonatal deaths. That is essentially true for the entire country, but if adequate post-mortem examinations had been carried out in all cases, a great number of cases would

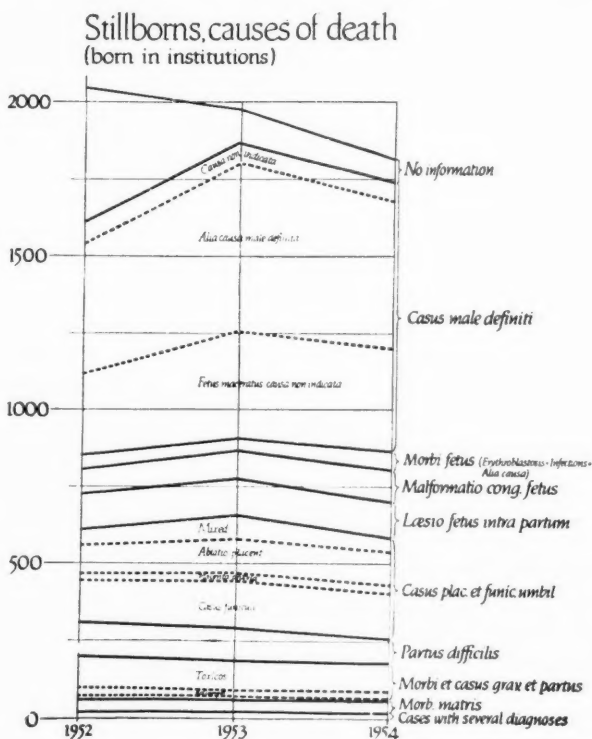


Fig. 1. Number of still-borns in Sweden during the years 1952—1954. Accumulatively grouped by different causes of death according to WHO's classification

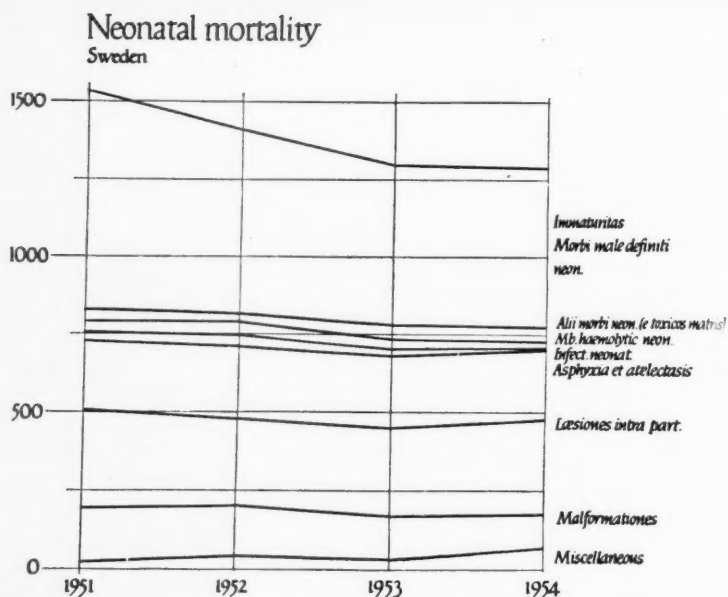


Fig. 2. Number of neonatal deaths in Sweden during the years 1951—1954 accumulatively grouped by different causes of death according to WHO's classification.

have been classified though even then with very uncertain diagnosis. This coincides with Dr. Ahvenainen's opinion as given in the discussion.

Little further will be gained if we divide up the cases according to the rough obstetric conditions, such as for example prematurity, colour of amniotic fluid, the cord around the neck, breech, forceps, section. We know that these conditions involve an increased risk for the child, but on the other hand is it not unusual that at such a delivery an alert, lively, well-crying child is born. Many of the perinatal deaths must include a very complex relationship between the various causes involved.

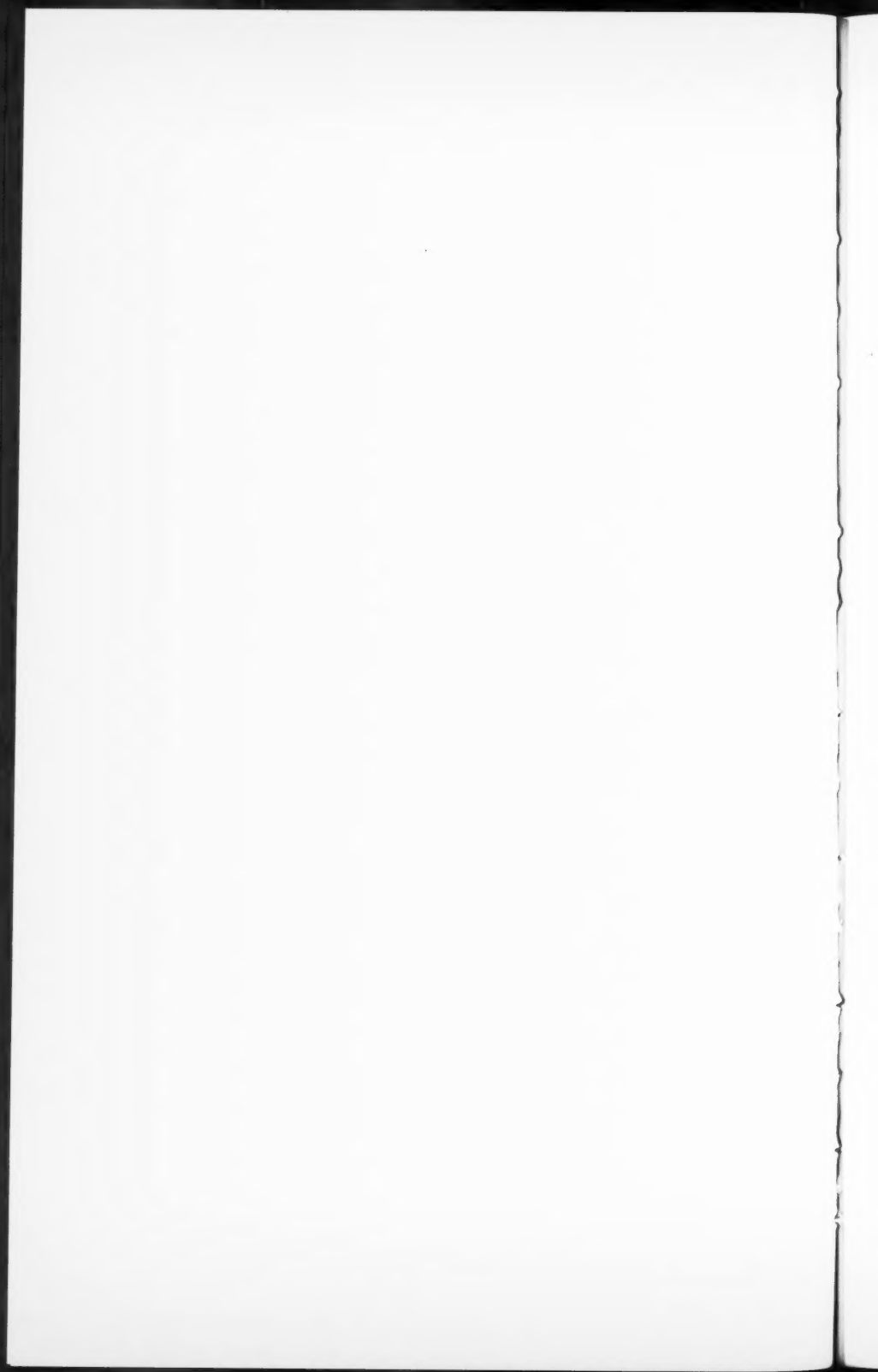
In my opinion, one way of increasing our knowledge of the causes of perinatal deaths would be a combination of careful clinical observation, obstetric as well as pediatric, well correlated with regard to time, and a thorough postmortem examination. A pilot study following these lines has already been started at Karolinska Sjukhuset, Södra BB and Falu Centrallasarett. This project is a part of a co-ordinated international perinatal study outlined by WHO.

*C.-E. Räihä, Finland.* — Prematures have more malformations and the babies born at term fewer in Lundström's material. Is there something suggesting

a normalizing induction by normal tissues during a longer intrauterine life which affects the availability of malformations for diagnosis (something that in some respects corresponds to a varying expressivity).

*R. Lundström, Sweden. —*

*J. Ström, Sweden. —* On the question of the significance of social factors for prematurity, and with it perinatal mortality, it can be said first, that social conditions are on the whole very good in our northern countries and that poverty, distress and various stresses hardly have a more marked influence. There is, however, another circumstance I should like to mention. In an investigation conducted in Sweden a few years ago it appeared that mortality of the children of unmarried mothers, provided they were born at term, was lower than that of children born in wedlock (Thorén). This depends on the fact that the unmarried mother is on the whole better off medically, both obstetrically and pediatrically, because of her younger age. On the other hand, unmarried mothers have a much greater percentage of premature babies, the percentage increasing with the degree of prematurity. Hence perinatal mortality is higher among the babies of unmarried than of married mothers and this ratio between the two categories of children has, as I have shown, become increasingly pronounced in Sweden during the last few decades. An artificial factor must be involved here; the phenomenon can hardly be explained on biological grounds. The question is surely one of induced abortions, and I want to emphasize here the importance of taking this into consideration in the assessment of prematurity and perinatal mortality. It is also important not to forget anti-abortion work as a significant aspect of our prophylactic work.



## SESSION II



## **Circulatory Adaptation at Birth**

F. ADAMS, P. KARLBERG and J. LIND  
Stockholm, Sweden

Studies of the circulation of the newborn, including pressure measurements in the umbilical blood vessels, catheterisation data, determinations of cardiac volume, electrocardiograms and phonocardiograms.

## **The Lung Function during the First Minutes of Life**

P. KARLBERG and G. KOCH  
Stockholm, Sweden

(to be published elsewhere)

## **Capillary Erection in the Living Lung**

S. JÄYKKÄ, TUOMAS PELTONEN and LEO HIRVONEN  
Turku, Finland

According to the capillary erection hypothesis, the filling of pulmonary capillaries plays an important role in pulmonary physiology. A dead lung preparation expands when the liquid pressure in the pulmonary artery is increased (Jäykkä 1956). The aim of the present study was to show if similar changes can be observed in living lungs. Changes in the pulmonary circulation were produced by intravenous administration of Indian ink (Sternberg and Tamari 1938), and by stressing animals in a revolving drum. Eight rabbits and 54 albino rats were used as the experimental animals. Immediately after killing the rats, the lung volume was determined according to the Archimedean principle, by weighing the lungs both in water and in air. In the rabbits, the blood pressure changes in the right ventricle and aorta were recorded with Sanborn manometers during Indian ink injection. The lungs of the various animals were studied microscopically.

The lung volume of the Indian-ink treated rats was smallest (2 to 3.8 cc.), and that of animals stressed moderately was greatest (5.5 to 11.8 cc.); the lung volume of the control rats was 4 to 5.5 cc.

The aortic pressure of the rabbits fell considerably and the right ventricular pressure remained unchanged or rose after Indian ink injection. With a moderate dose, the blood pressure changes were reversible.

In the microscopical examination, atelectatic areas, contracted arteries and corrugated capillaries were found in the Indian-ink treated lungs. The lungs of the runnings animals were well expanded with opened arteries and filled capillaries.

The findings were explained as being due to a neurovascular function or capillary erection.

### **The Oxygen Tension of the Amniotic Fluid during Oxygen Administration to the Mother\***

SVEN SJÖSTEDT, GÖSTA Rooth and FRANCO CALIGARA  
Lund, Sweden

An analysis of the perinatal deaths at the department of Obstetrics and Gynecology, University Hospital Lund, shows that, in the years 1948 to 1957, fiftyfive per cent of the infants died before or during delivery, and 45 per cent after delivery. The cause of death in many of the infants who die after delivery is to be found in abnormal praenatal conditions and, according to CROSSE and MACKINTOSH (1954), it is only in 12.4 per cent of the infants who die in the postnatal period that the cause of death is primarily postnatal. The true causes of death are unknown in a large number of the cases of perinatal death, a fact which is specially true of intrauterine deaths, where the foetuses are macerated. In our hospital this group amounts to 38 per cent of the perinatal deaths.

Those infants who die before delivery will, before they have died, have shown signs of disease which, if it had been possible to observe and interpret them rightly, might perhaps have led to a correct diagnosis and therapy.

Unfortunately, our means of observing the foetus are very limited. Our most important observation, changes in the rate of the foetal heart, is usually noted only when the foetus is in acute and serious danger. Methods such as foetal ECG, X-ray or transaminase determinations of the mother's blood or maternal urinary pregnandiol excretion all have very limited value.

In order to try to obtain information from the foetus, we have measured the oxygen tension of the amniotic fluid.

\* This study has been supported by grants from the Association for the Aid of Crippled Children, New York.

*Material:* A total of 54 cases were analysed. 28 cases were induced abortions where the uterus was punctured from the vagina. In 26 cases the mothers were near term and the amniotic sac was punctured through the abdominal wall or during Caesarean section.

In 5 cases we inserted a plastic catheter after puncturing the amniotic sac and then intermittently drew samples for analyses.

In the meanwhile the mother was given oxygen, usually with RADNER's nose catheter (1949). In this case the arterial oxygen tension of the mother rose from 100 mms Hg to 200 or 250 mms Hg. Sometimes the oxygen was given with a face piece and then the arterial oxygen tension of the mother rose to 400 to 600 mms Hg.

In order to obtain another intra-abdominal oxygen tension for comparison ten benign ovarian cysts were punctured. Nine of these were follicular cysts and 1 was a lutein cyst. These punctures were all made during laparotomy.

*Technique:* The oxygen tension of the amniotic fluid is measured polarographically either with the dropping mercury electrode or with the Clark electrode, as described earlier by us (ROOTH, SJÖSTEDT & CALIGARA, 1958, SJÖSTEDT, ROOTH & CALIGARA, 1958). At least 2 ml of amniotic fluid are needed.

*Results and discussion:* The results are presented in Table I. The oxygen

TABLE 1  
OXYGEN TENSION OF THE AMNIOTIC FLUID AND OVARIAN CYSTS

Amniotic fluid	Number	Range among cases near term	Ovarian cyst	Number
11 mms Hg	54	2—15 (20)* mms Hg	45 mms Hg	10

tension in the ovarian cysts is about 45 mms Hg, which probably agrees with the intra abdominal tissue oxygen tension of the woman. The amniotic oxygen tension is considerably lower and in several cases so low that there is almost no oxygen measurable. The average oxygen tension is probably lower than the figure given as in some instances the samples might have been contaminated by the surrounding oxygen.

As we have shown in an earlier study (ROOTH, SJÖSTEDT & CALIGARA 1957) there is a rapid equilibration between the skin tissues and a surrounding fluid. Five ml of fluid at 45°C, in which a finger is immersed, will have the same oxygen tension as the tissues after 10 minutes. As the surface of the foetus which is in contact with the amniotic fluid is large and the time is long, the oxygen tension of the skin of the foetus must be approximately the same as that of the amniotic i.e. of the order of 10 mm Hg or less. The oxygen con-

\* One case (the last in fig. 1) is excluded because the foetus was dead in utero. The value 20 is probably caused by technical error.

*Oxygen tension of the amniotic fluid during oxygen administration to the mother.*

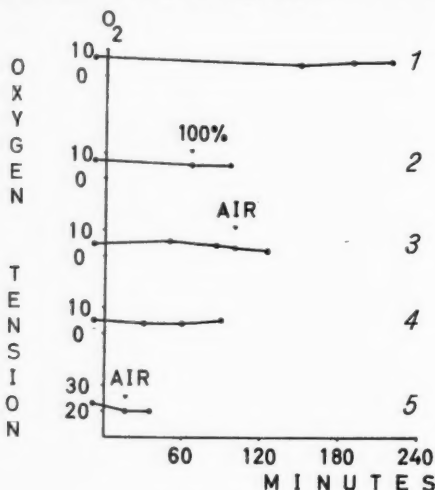


Fig. 1.

Oxygen tension in mms Hg.

The oxygen administration to the mother began at O<sub>2</sub>. The values left of this line represent the oxygen tension before the administration of oxygen. At 100% the mother was given oxygen with a face piece. At 'air' the mother began to breath only air.

sumption of the amniotic fluid is negligible. The influence of oxygen administration to the mother on the oxygen tension of the amniotic fluid is shown in Fig. 1.

In case No. 1 the mother was given oxygen for almost 4 hours and no increase was observed in the amniotic fluid. The same results were obtained in cases 2, 3 and 4. It seems from these results that oxygen administration to the mother does not increase the oxygen tension of the tissues of the foetus.

Case No. 5 gives some additional information. The foetus had died before the samples were taken, and it will be observed that the oxygen tension in the amniotic fluid in this case is higher than in the other cases. This indicates that the amniotic fluid receives some oxygen from the mother and that the living foetus absorbs some oxygen from the amniotic fluid. Consequently the oxygen tension of the skin of the foetus will not be higher than that of the amniotic fluid.

*Conclusions:*

1. The oxygen tension of the amniotic fluid and at least some of the tissues of the foetus is 10 mm Hg or less. It may often be about 2 to 5 mms Hg. This shows that the foetal tissues are able to extract almost all the oxygen available. The results may also support the opinion that the anaerobic metabolism of the foetus is important.

2. Some oxygen may be transported to the foetus from the mother via the amniotic fluid. The total amount carried this way is probably small and without practical significance to the foetus.

3. Oxygen administration to the mother does not increase the oxygen available to the infant. This is also recognised from theoretical considerations. As the arterial blood of the mother is fully saturated with oxygen, an increase in oxygen tension will give only a small increase in the physically dissolved oxygen and only an insignificant increase in the total amount of oxygen carried. If, on the other hand, the mother is hypoxic, oxygen therapy may raise the oxygen tension in the umbilical vein to a considerable extent.

4. It is not yet known whether the measurement of the oxygen tension of the amniotic fluid is of any practical importance in the management of deliveries. In our cases, this tension varied from 2 to 15 mms Hg. It is possible that further studies will reveal that chronic hypoxia of the foetus may be diagnosed in utero by this method and that proper therapy — induction of labour or Caesarean section — may be instituted in time. We would then have a means of guiding cases of dysmaturity or other placental insufficiencies.

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#### DISCUSSION

C-E. Riih , Finland. — A falling  $O_2$  tension during foetal life, mentioned by the author, perhaps supports the investigation by Walker & Turnbull.

S. S st d, Sweden. — During the early months of pregnancy the  $pO_2$  of the amniotic fluid seems to be higher than at term. However, the material is not big enough for definite conclusions. It is not justified to use our findings as a confirmation of Walker & Turnbull's hypothesis. Furthermore their findings have not been confirmed by several authors in different countries all having much larger material. Lastly, Walker now only maintains that the decrease in oxygen saturation with advancing gestation time only occurs in primigravidae.

## Some Characteristics of the Blood Plasma Chemistry in Newborns

GUNNAR CHRISTIANSSON

Stockholm, Sweden

In an investigation which was carried out in order to establish certain normal values in the blood in children, special attention was paid to the newborn. In the first place, studies were made of the enzymatic conditions which have, in the past few years, attracted a good deal of interest in most other medical fields. In addition, some other data referring to the chemistry of the blood were registered as being of significance in that connection. For the sake of comparison, corresponding analyses have, in some instances, been carried out on older healthy children up to the age of 7 years, and on the mothers at parturition.

The investigation comprised alkaline serum phosphatases, serum glutamic oxaloacetic transaminase (SGO-T), serum glutamic pyruvic transaminase (SGP-T), lactic dehydrogenase (LD), bilirubin and uric acid.

The following summarized conclusions can be drawn from this:

1. The phosphatase activity in newborns is strikingly low as compared with other age-groups and the mothers.
2. The SGO-T activity shows a higher mean value during the first year of life with a more marked deviation and uncertainty during the first week of life.
3. The SGP-T and LD concentrations in newborns, in a small material, show approximative agreement with those in adults.
4. As regards the SGO-T, SGP-T and LD concentrations, there is no ascertainable correlation with the degree of physiologic bilirubinemia.
5. The concentration of uric acid is significantly higher in newborns. This may possibly be attributed to the cellular transformation in the blood during the newborn period.

### DISCUSSION

*S. Sjöstedt*, Sweden. —

*G. Christiansson*, Sweden. — We have had no major troubles in taking samples from the umbilical cord. As was mentioned by way of an introduction, we deliberately selected only normally delivered, apparently healthy, full-weight infants. Hence there was no current interest in this material to correlate the results obtained with the baby's condition on birth.

## Treatment of Premature Infants with 15% Oxygen

G. ENGLESON, G. ROTH and S. SJÖSTEDT

Lund, Sweden

An increased oxygen tension in premature and newborn infants produces important vasomotor reactions in the vessels of the brain, lungs and eyes. Retinal and cerebral vessels are constricted and the vessels of the lungs are dilatated. A decrease in oxygen tension causes opposite reactions in these organs.

As is well known, premature infants have a tendency to develop retrolental fibroplasia, hyaline membrane and cerebral palsy. Almost all pediatricians are now of the opinion that the development of retrolental fibroplasia is due to the increased oxygen tension caused by the administration of high concentrations of oxygen. As a matter of fact this treatment has also been abandoned in most countries.

As to hyaline membrane disease, it has been shown experimentally that oxygen increases the blood volume in the lungs and also the development of hyaline-like membranes.

Some authors have stressed the coexistence of retrolental fibroplasia and cerebral palsy in premature infants.

On the basis of these findings, we have found it logical to treat premature infants in incubators with an atmosphere of low oxygen content, i.e. 15% oxygen and 85% nitrogen.

At first, newborn rats were kept in cages with this mixture (Rooth & Sjöstedt). No side-effects were obtained, and later the method was applied to newborn premature infants.

Immediately after the infant was born, the airways were cleared by means of a resuscitator; subsequently, the newborn infant was placed in an airtight incubator through which a mixture of 15% oxygen and 85% nitrogen was blown at a rate of 5 litres/min. Oxygen tension was checked by means of a Beckman D oxygen analyser. The temperature was about 29°C (the infant lying naked) and the relative humidity about 75%. The infants were fed by means of a continuous milk drip — at first only breast milk; later on a small amount of citric acid milk was added — via a polyethylene tube.

Up to now some 50 newborn infants have been treated, 25 of these being premature infants with a birthweight between 520 gr to 2,200 gr.

When a newborn full-term infant is placed in the incubator with 15% oxygen it usually falls asleep. No cyanosis develops; on the contrary breathing is deep and quiet, the heart rate is rather slow.

A newborn premature infant behaves like a full-term infant. During the

first 2 or 3 days, however, some respiratory difficulties can be seen with small attacks of cyanosis and dyspnoe; we have a distinct feeling that these attacks are less dangerous and rarer than usually happens among premature infants.

After the third day, these symptoms disappear and the newborn premature infant behaves precisely as a full-term infant, i.e. it sleeps most of the day and night, the respiratory rate is about 45/min. and the heart rate about 100—110/min.

The temperature is low — about 33°C — during the first few days, but increases gradually to about 36°C on the 10th day of life.

Our experience during 2 years treatment have so far been favourable. Weight increase and general condition have been very good. On 2 occasions one of a pair of twins was treated in the incubator with 15% oxygen and the other in room air. In both cases the infant in 15% oxygen — with the lowest birth-weight — was in better condition, and on later examinations general development was faster and more advanced for these infants.

All premature infants have been examined at regular intervals, and we have the impression that most of these infants have been physically and mentally more advanced for their age during their first year of life. This impression has been confirmed by other observers.

Ophthalmological examinations have been made by a specialist, and no case of retrolental fibroplasia has occurred. In fact, some observations speak in favour of a dilatation of retinal vessels.

Electroencephalographic and electrocardiographic examinations have been entirely normal.

From experimental experience (Rooth and Sjöstedt), there were some reasons to find a decrease in the frequency of prematurity anemia. Our present experience is that most of the treated premature infants develop anemia, but this is less pronounced than is usually found. In our experiments with twins the infant treated with 15% oxygen had less anemia than the infant treated in room air.

No serious side effects have occurred. 5 patients died; the first case had a birthweight of 520 grams, one case had multiple malformations and in 3 cases prematurity was combined with other complications, i.e. birth trauma, toxemia, placenta previa and so on.

Treatment with 15% oxygen should for the present not be used as a routine method.

#### DISCUSSION

*P. Karlberg, Sweden.* — I find this therapeutical programme very interesting. From having until a few years ago regarded ourselves as helping the premature through a generous supply of oxygen gas we have now come to the conclusion that a lower oxygen concentration than in the air increases their chances of life.

I should like to put two questions:

What do you do if the premature baby has an attack of cyanosis while it is in an atmosphere with 15% O<sub>2</sub> (which occurs, according to Dr. Engleson)?

The therapeutical schedule involves keeping body temperature 2—4° below 37°. Can this hypothermic treatment have contributed to the results attained?

*C.-E. Råihä, Finland.* — My congratulations on both the interesting experiments. Is there anything to indicate what happens with this treatment; whether it is the hypothermy which reduces the need for oxygen or the low oxygen tension which produces hypothermy?

*G. Engleson, Sweden.* —

## **Respirator Treatment of Pulmonary Insufficiency in the Newborn**

F. BENSON and O. CELANDER

Göteborg, Sweden

Neuromuscular block and positive-pressure respirator treatment has been tried in cases of life-threatening respiratory distress in new-born infants. A diagnosis of pulmonary insufficiency has been based on the clinical appearance of the child (a marked increase in respiratory frequency being considered the most important single symptom) as well as the radiographs of the lungs, and, in most cases, determinations of CO<sub>2</sub> tension and pH of capillary blood. Only when the child has been evidently deteriorating has the respirator treatment been considered. In some cases, endotracheal intubation and manual artificial respiration has been an emergency measure in children dying from pulmonary insufficiency. Tracheotomy and respirator treatment has been the logical continuation of this initial resuscitation. Neuromuscular block has been used both to lower body temperature and skeletal muscle activity. This will cut down the metabolic activities of the body, and therefore decrease the demand for a respiratory gas exchange making the pulmonary insufficiency less prominent. In addition, blocking of the child's own respiratory efforts makes the adjustment of the respiratory volumes easier. The positive pressure necessary will also be lower and the risk of pulmonary rupture less.

Of our first nine cases, three have been successfully treated for periods up to 13 days, making a complete recovery. Four prematures died shortly after the start of respirator treatment, and on autopsy showed almost total atelectasis of their lungs. One child died suddenly on the fifth day of treatment and on

autopsy there was extensive bronchopneumonia. The last child of this series died unexpectedly and on autopsy showed partial atelectasis of the lungs, hyaline membranes and a small pneumothorax.

#### DISCUSSION

*S. Jäykkä, Finland.* — I would like to contribute to our knowledge of circulation conditions in the lungs with a few anatomical data:

According to v. Hayek, there is a »short circuit» past the capillaries. It travels via pulmobronchial anastomosis and flows into the venous plexus of the bronchus under the mucosa. If Indian ink is injected into the pulmonary artery in a »respiratory distress» lung it can be seen to travel via v. Hayek's short circuit» in the atelectatic parts of the lung. It seems to me that theoretically artificial respiration is hardly beneficial for impaired diffusion when the capillaries are not perfused. Ventilation may be justified when the child has apnoea. I would, however, prefer for theoretical reasons Ylppö's old method of applying oxygen via the digestive tract.

### Infection of the Foetus during Labour

LARS ENGSTRÖM and BIÖRN IVEMARK  
Stockholm, Sweden

The purpose of the study was to investigate from clinical, bacteriological and pathological points of view the infections transmitted from the vagina to the foetus during labour.

The material — 138 delivery cases — was selected at random.

The following problems were studied:

1. The influence of early membrane rupture and prolonged labour on the occurrence of infections among the infants.
2. The composition of the bacterial flora.
3. The value of prophylactic and therapeutic use of chemotherapeutics and antibiotics.

Samples for bacteriological examinations were taken from the throats of the infants at birth, from umbilical cord blood and from placenta tissue. The placentas and cords were examined histologically.

The following conclusions were made:

Pathogenous bacteria (mostly coli and enterococci) were often present in the infants' throats at birth without any clinical signs of infection during the neonatal period.

Inflammation of the umbilical cord and the placenta occurred in 2% if labour started and membranes ruptured within 24 hours before delivery (normal deliveries). In cases with rupture of the membranes earlier than 24 hours before delivery, inflammation was seen in 26% even if the labour pains lasted less than 24 hours. On the other hand, inflammation was found in 38% in cases of prolonged labour, but with rupture of the membranes within 24 hours before delivery. This means that bacteria can infect the foetus in spite of unruptured membranes and reach the uterine cavity in cases without uterine contractions but with rupture of the membranes. From the obstetrical point of view it is of great importance to induce labour when the membranes are ruptured in full time and to shorten labour in cases of inertia.

Before determination of type and resistance of the bacteria, the routine employment of chemotherapeutics and antibiotics is of doubtful value.

### **Paroxysmal Atrial Tachycardia and Atrial Flutter in the Neonatal Period**

ÅKE LUNDBERG  
Stockholm, Sweden

The clinical material consisted of 30 cases of paroxysmal atrial tachycardia and 7 cases of atrial flutter in infants from Swedish Childrens Hospitals. The diagnosis was confirmed by ECG during the acute attack.

Four paroxysmal tachycardias and 4 flutters were diagnosed during the neonatal period. In half of these cases, the arrhythmia was suspected before birth, of which 3 were seen to have flutter after birth. Most of the flutters amongst the infants commenced in the neonatal period, which might suggest that the cause lies in an immature conduction system. Treatment with digitalis, preferably as digitoxin with its more prolonged action has given good results per os.

Recurrences during the first month of life have been observed in 2 of the cases of paroxysmal tachycardia but in none of the flutters. The prognosis has been good in every case and no accompanying disease has been found.

## Indications for Exchange Transfusion in Icterus Neonatorum

ANDREAS KILLANDER and STIG SJÖLIN

Uppsala, Sweden

The toxic effect of indirectly reacting bilirubin is well known, and in infants with haemolytic disease due to Rh-immunization the indications for exchange transfusion are established. However, opinions differ as to the treatment of other icteric newborns. In some centres, the hyperbilirubinaemia of premature infants is treated by exchange transfusion, but the indications vary.

To be able to define more clearly the indications for exchange transfusion in cases of hyperbilirubinaemia not due to Rh-immunization, we investigated all newborn infants in Uppsala in 1957. The series studied comprised 88 premature and 1987 full-term infants, who were observed with respect to the occurrence of hyperbilirubinaemia and neurological symptoms.

Exchange transfusion was carried out on the 8 premature infants reaching a serum bilirubin level of 20 mg%, and in 3 additional infants who showed neurological symptoms at a bilirubin level of 15–20 mg%. Most cases with neurological symptoms showed a marked improvement after the treatment. No case of kernicterus was noticed, in contrast to the findings in 1954 and 1955, when 6 premature infants died of kernicterus.

The previous finding of kernicterus in a few full-term newborns initiated a study of the use of exchange transfusion also in full-term newborns. Exchange transfusion was performed alternately on 53 infants reaching a serum bilirubin concentration of 20 mg%. A comparison between the two groups showed an earlier reduction of the serum bilirubin in the treated group. No differences were noted between the two series as regards their clinical condition immediately, or after 3–4 months. In 14 of the 53 patients, the hyperbilirubinaemia was probably due to A- or B-immunization. These infants did not differ from the others with regard to the degree of hyperbilirubinaemia, the onset of icterus or the occurrence of symptoms.

From the preliminary result, we suggest that exchange transfusion should be performed on premature infants attaining a serum bilirubin level of 20 mg%, and also on those with neurological symptoms at a lower concentration. In full-term newborn infants, exchange transfusion should be restricted to cases with excessive and early hyperbilirubinaemia (25–30 mg%).

### DISCUSSION

*P. Plum, Denmark.* — Retrospective investigations into the incidence of severe icterus neonatorum, with and without sensibilisation, in patients with

athetosis have shown that kernicterus without sensibilisation seems to be fairly common both in premature babies and in those born at term. (See the Ylppö Anniversary Publication 1957).

*G. Christiansson, Sweden.* — We consider bilirubin determinations on the newborn a difficult task as venous blood is needed in relatively great quantities for repeated determinations. In the Kronprinsessan Lovisas Barnsjukhus a micromethod has been tried for the analysis. Parallel determinations with this method and with the customary macromethod, however, showed very poor agreement and we therefore felt obliged to abandon the former. It would be valuable to know what method Docent Sjölin employed for these, if I understood him correctly, daily determinations.

*M. Skatvedt, Norway.* — We have found no incompatibility of blood groups between mother and child in nearly a half of our kernicterus cases in the cerebral palsy material from the Rikshospitalet in Oslo. It seems to me a daring measure to allow bilirubin values to rise as high as 25–30 mg% since kernicterus has been seen in prematures with bilirubin values of 17–18 mg%. It is not possible to diagnose cerebral damage in medium-severe and mild cases until considerably after the age of 3–4 months. Athetosis is not found until the age of 8–9 months at the earliest, and impaired hearing and mental subnormality even later. To wait until neurological symptoms appear in an icteric infant may be to wait too long. The cerebral damage may already have occurred.

*P. W. Bræstrup, Denmark.* — The risk of damaging the child through repeatedly taking large quantities of blood for bilirubin determinations can be reduced if primary serum dilution is used for the analysis. This method gives uncertain results for normal values but reasonably reliable results when the bilirubin quantities fall within the range of actual interest, that is around 20 mg%.

*S. Sjölin, Sweden.* — The serum bilirubin concentration has been determined according to Jendrassik and Grof, using 0.2 ml of serum. Whole blood has been obtained by a deep heel puncture. The importance of warming the foot is stressed. In order to avoid haemolysis, only specially trained nurses should be allowed to take the samples.

We should like to stress that in assessing the need for exchange transfusion not only the serum bilirubin level should be taken into account, but also the occurrence of even slight neurological signs.

The final follow-up study on these patients is planned to be carried out at the age of 2 years.

## A-B-O Sensitization in the Newborn. Diagnosis, Frequency and Symptoms

HOLGER DYGGVE and GEORG MUNK-ANDERSEN

Copenhagen, Denmark

Two methods for the demonstration of anti-A or anti-B sensitization of the red cells have been employed for the examination of cord blood from 3,500 newborn infants. The methods were Rosenfield's modification of Coombs direct antiglobulin test (*Blood* 10: 17, 1955) and Munk-Andersen's direct conglutination test (*Acta path. et microbiol. scandinav.* 38:259, 1956).

In 71 cases (two per cent), all type A or type B infants of type O mothers, the red cells of the infants were found to be sensitized by A-B-O antibody. The serological diagnosis was confirmed by elution of antibody from the red cells, and demonstration of the specificity of the antibody. In the mother's serum incomplete immune anti-A or anti-B could always be demonstrated, whereas no other immune antibodies were found.

Sex distribution, birth weight, complications of pregnancy and labour and the outcome of earlier pregnancies has been analysed. One third of the cases occurred in primigravida. 13% of the infants were premature. The two smallest premature infants died without being icteric. Often a considerable weight loss was observed. 42% of the infants became icteric before the age of 48 hours. While no visible jaundice was observed in one fourth of the infants, one third became severely jaundiced, and 11 infants reached serum bilirubin levels above 20 mg%. Haemoglobin values were lower in the A-B-O infants than in 150 controls, but no case of severe anaemia was seen. Reticulocyte counts were higher in the affected infants than in the controls.

Only two infants received exchange transfusions with type O blood and the addition of AB-substance. While none of the infants had definite symptoms of kernicterus during the neonatal period one of them has shown such signs at the follow-up (which is still in progress). It is concluded that more exchange transfusions should be given in the future to prevent kernicterus. Our experience suggests that exchange transfusions on account of A or B sensitization will be necessary in about three pro mille of all newborn infants.

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### SESSION III

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## Congenital Malformations of the Urinary Tract. I. Diagnosis

JÖRGEN VESTERDAL  
Copenhagen, Denmark

In this and the following paper (by Dr. Winkel Smith) we will present a case material, consisting of the urological patients admitted to the University Clinic of Pediatrics, Copenhagen, during the years 1949—1958.

This series comprises 183 cases submitted to urological examination. In this number there are not included cases of simple hypospadias, pseudohermaphroditism and enuresis.

The indications for urological examination were:

- 1) recurrent or persisting pyuria (a single attack of pyuria does not, in our opinion, necessitate a thorough examination).
- 2) retention of urine, frequency, dribbling or incontinence. In common enuresis we have only in part of the cases taken urograms; these were all normal and have therefore not been included in the present series.
- 3) hematuria (except in acute nephritis and hemorrhagic diathesis).
- 4) abdominal colic, with a localization suggesting a urinary disorder or with uncharacteristic localization.
- 5) palpable mass in the kidney region or in the lateral part of the abdomen.
- 6) renal insufficiency of unknown origin.
- 7) hypertension.
- 8) anomalies of the external genitals. Cases of this type have not been included in the present series. We have seen 14 cases of adrenogenital syndrome, 7 cases of masculine pseudohermaphroditism, and 72 boys with uncomplicated hypospadias; urograms were taken in 36 cases of the last group; the result was normal in 32, while 4 had anomalies without any symptoms (3 had double kidney and 1 had fused kidney).
- 9) passing of calculi.
- 10) «neurogenic bladder» in myelomeningocele and similar conditions where surgical intervention is justified to relieve the symptoms.

The following procedures were carried out: intravenous (or intramuscular) urography — it may be difficult to obtain good pictures in small infants owing to the small concentrating capacity of the kidney and to air in the intestines overlapping the kidneys; the latter impediment may be avoided by giving the infant sodium bicarbonate by mouth, so that the air-filled, distended stomach presses the intestines down and permits a clear view of the urograms. Furthermore we carried out cystoscopy, retrograde pyelography, urethroscopy, cystography, urethrography, and serial cystograms of micturition. The latter technique proved very useful, as it might yield information on reflux into the ureters

and on the function of the internal sphincter and the adjoining part of the bladder-during micturition.

In almost half of our cases the symptoms started at birth or in the first year of life. In small infants, vomiting and failure to thrive were predominant symptoms.

The results of the examination of the 183 patients were as follows: in 17 cases with recurrent attacks of pyuria, no anatomical anomaly could be demonstrated; in the remaining 166 cases the most frequent finding was one or more obstructions of the passage in the urinary tract, with secondary dilatation of the proximal parts and recurrent infections. Several patients had more than one disorder. The findings are summarized in table 1.

TABLE 1  
FINDINGS IN 166 PATIENTS WITH UROLOGICAL DISEASES

Malformations of the kidney .....	40
— of the ureters.....	55
— of the bladder and urethra .....	55
Hydronephrosis of unknown origin .....	9
Calculi .....	13
Tumours .....	22
Incontinence due to myelomeningocele, etc. ....	8
Total	202

Among malformations of the kidney the most frequent was duplicated pelvis, which was seen in 19 cases. In 14 out of these the ureter was also duplicated. This type of malformation occurs not infrequently without any symptoms. When symptoms arise, the cause is obstruction of the urine flow; this occurs most frequently in the ureter from the upper segment of the duplicated kidney, very often with the formation of a ureterocele in the bladder wall. In one case, there was found an ectopic ending of one part of a duplicated ureter into the vulva. In some cases one of the segments of a duplicated kidney was not functioning, and this might cause great diagnostic difficulties.

Stenoses in the ureters were found in 34 cases, situated either proximally near the pelvis of the kidney or, more frequently, distally, near or in the bladder wall, often with a ureterocele.

Dilated and tortuous ureters were in most cases secondary to obstructions in the lower urinary tract. No such obstruction could be demonstrated in 4 girls with primary megaloureters. In this condition, which seems to be due to a neuromuscular disturbance, the ureters are enormously dilated, with a wide and funnel-shaped lower orifice. These patients have much residual urine because during micturition the urine flows up into the ureters and returns to the bladder when the micturition is finished.

Among the malformations in the bladder and urethra the most important are various hindrances to the urine flow from the bladder. 11 cases had valves in the urethra. In 14 cases sclerosis of the internal sphincter was found, and in ten cases there was great suspicion of this disorder. These patients have difficulties in emptying the bladder, with residual urine, trabecular bladder and, frequently, secondary dilatation of the upper parts of the urinary tract. By means of cystoscopy a pillow-like thickening of the bladder wall in the trigonum is seen, and serial cystograms during micturition show that the bottom of the bladder does not change its form during micturition (normally it becomes funnel-shaped), so that one gets the impression that the tissue is hard and sclerotic.

In most of the 13 cases of calculi the stone formation was secondary to a malformation with chronic infection.

Tumours have been included in our case material. 17 cases had Wilm's tumour. In two thirds of these, the first symptom was a palpable mass in the abdomen or the loin. As this tumour is very malignant we do not waste time on too many examinations, but operate upon the child as soon as there is a fair suspicion of this disease. In spite of this, one was inoperable, 3 died and 6 had metastases or ingrowth into adjoining tissues.

Finally, we have included 8 cases with severe incontinence (in 7 cases due to spina bifida with myelodysplasia and in one case for unknown reasons). These patients were socially incapacitated to such a degree that we felt that rather extensive surgical action was justified to relieve the symptoms.

## **Congenital Malformations of the Urinary Tract**

### **II. Surgical Treatment**

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A complete survey of the surgical treatment of the congenital malformations of the urinary tract would comprise more than 20 different groups of surgical problems and procedures. As it is impossible to give this account within the allowed space of time I have chosen a few major subjects, where the current surgical achievements have been found of special importance to pediatricians in their daily dealing with sick children.

*Obstructions of the ureteropelvic junction.* The material of organic obstructions includes 21 patients who were operated upon 27 times in all. In 3 cases a primary plastic operation was followed by nephrectomy, 3 patients had a bilateral plastic operation done, and in 1 — a girl with one kidney — an insufficiently

functioning plastic operation was followed by a ureterectomy substituted by an isolated ileum loop.

Thirteen of the 21 patients were cured by conservative plastic procedures (Y--V-plastic in 4, Davies' ureterotomy in 8 and resection in 1 case).

Two elementary factors are of the greatest importance for good results in urologic surgery: 1) early diagnosis, if possible already in early infancy before the obstructive lesion has caused irreversible parenchymal damage to the kidney and 2) the correct choice of surgical technique.

In this material, Davies' intubationureterotomy for obstructive lesions in the upper part of the ureter has proved to give excellent results in 8 of the 11 cases in which it was used. The other recent advance in urosurgical technique is the use of an isolated ileum loop as a substitute for either a part or the whole of the ureter. In the present material, this technique has been used twice with very promising results. Although too early to be definitively assessed, the ileum loop surgery in urology must be brought into consideration in cases with badly functioning ureters. It might well be a life-saving procedure.

*Duplication of the kidney and the ureter.* From a clinical point of view this problem has turned out to be of much greater importance than realized before, first of all due to better diagnostic methods. Cysto-urethrography and cystoscopy in early infancy and childhood have made it possible to detect these anomalies, which very often are impossible to demonstrate urographically. A great number of the ureters are ectopic, often combined with a ureterocele either inside the bladder or in the urethra.

In the present material 20 cases of bilateral duplications were found. Only 3 of the patients were boys. This corresponds to the figures of Stephens from Australia, who found 4 boys out of 28 cases. Half of the cases in our material were bilateral and in 15 a dilatation could be shown, either in the lower part or in the upper.

It is a well established fact that heminephro-ureterectomy is the technique of choice for duplication, whereas dilatation of the stenotic orifice or splitting of the ureterocele alone are inadequate procedures. Splitting was tried in 4 cases. None of these children were cured, in 3 cases the condition improved while one had symptoms as before. In 9 cases a heminephro-ureterectomy was performed. Eight of these children were completely cured while one child had a slight, persistent pyuria. A postoperative excretory urogram showed excellent function of the remaining part of the kidney. In all cases the dilated part of the kidney was resected. In 2 cases a unilateral nephrectomy was necessary, after which both children are doing well.

*Anomalies in the lower urinary tract* is a very complicated subject to deal with because of the still many unsolved diagnostic and therapeutic problems. Four groups of organic lesions must be considered: 1) bladder-neck-obstruction,

2) urethral valves and hypertrophia of the verum montanum, 3) stenosis at the uretero-vesical junction and 4) primary megaloureteres. 2 clinical entities may further be added to these 4: 1) patients with recurrent acute cysto-pyelitis where no organic lesion can be demonstrated with our present methods of examination and 2) patients with a chronic urethritis causing a persistent infection in the urinary tract.

The clinical picture in bladder-neck-obstruction differs very much in boys and girls, being of a more serious nature in the former. In our material it was found in 10 girls. As therapy, a transurethral resection was performed in all cases, after which 5 were cured. 3 cases had later on a transvesical sphincterotomy, apparently with good effect.

Nine boys had bladder-neck-obstruction, 6 infants and 3 bigger boys. How serious this anomaly is can be judged from our material: 1 died untreated in the neonatal period while 4 died at a few years of age in spite of a thorough treatment including cystostomy, sphincterotomy and nephrostomy. Although the prognosis seems to be poor it might be improved if the children are admitted for treatment at an early stage.

*Megaloureters.* One of the unsolved urological problems is whether megaloureters constitute a clinical entity per se or not. Personally I believe so. We meet cases with very little infection, with pathologically big openings into the bladder, where profuse vesico-ureteral reflux is demonstrated at the X-ray examination, and where an enormous hyperperistalsis and antiperistalsis can be seen in the ureters during the operation. The treatment of these cases is still not definitively settled. In some cases antibiotic treatment will be quite enough to keep these children free from symptoms. In more severe cases a resection of the ureter may be tried. In the present material this was carried out in two cases, bringing both children in a much better clinical condition.

*Urinary incontinence.* The treatment of urinary incontinence is one of the most difficult surgical problems in urology. On the other hand a constant leak of urine is so invalidating for the patient that the state of affairs must be changed.

Three surgical procedures different in principle are at hand: 1) ureterocolic anastomosis 2) Bricker's isolated ileumloop-bladder and 3) Lowsley- Johnsons isolated rectum-bladder with trans-sphincteric colostomy. With ureterocolic anastomosis the urine and feces are mixed, whereas they are held apart in the ileumloop- or rectum-bladder procedure, a circumstance which has proved to be of the greatest prognostic importance.

ad 1) Recent years have shown an increasing criticism of the basic principle in ureterocolic anastomosis, based on the fact that hyper-azotæmic acidosis develops in a number of patients thus operated upon. Our own experience with thirteen patients corresponds to that of several others, being that about 2/3 of

these patients will eventually develop hydronephrosis and suffer from recurrent infection and changes in the biochemical balance. On the other hand some children do perfectly well with ureterocolic anastomosis, and in my opinion it is too early to condemn this operation completely.

ad 2) Bricker's isolated ileumloop-bladder is — as far as I can see — an excellent operation particularly for children with meningocele where there is also an insufficiency of the anal sphincter. 10 children are doing very well after this operation. It is amazing to see how little these patients are inconvenienced by their urine-bag.

ad 3) the Lowsley-Johnson procedure is still a rather new technique but very interesting and promising. Its great value lies in the fact that the urine and the feces are diverted, both being under the control of the anal sphincter.

My personal experience with this procedure is still very small as I have only used it in 3 cases, but the results so far have been promising. The anal sphincter is functioning well in spite of the intrasphincteric operation, as can be judged by the satisfying continence of urine in the rectum. The difficulties lie in achieving normal voiding reflexes in the transplanted sigmoid.

### **Bacteriological Findings and Resistance to Antibiotics in Chronic and Recurring Urinary Tract Infections in Childhood**

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Urinary tract infections are very common in pediatric practice. Today, most of the acute infections can be readily cured by medical treatment, but there are still many problems concerning the therapy of the chronic and recurring infections, and the introduction of modern chemotherapeutics and antibiotics has not simplified these problems to any considerable degree.

A series of cases from the Childrens Ward, Ullevål Hospital, Oslo, is presented. 108 children, 89 girls and 19 boys, were treated in this department in the period 1950 to 1957 for chronic or recurring urinary tract infection. 24 of the patients have been admitted to the ward two or more times, and altogether they represent 148 admittances.

The sex distribution shows the usual great predominance of girls (in this series more than 82 per cent), as well as the equal sex distribution in the first year of life, with 12 girls and 10 boys. In most cases the symptoms started during the first 2—3 years of life, and most frequently before 1 year of age.

Urinary tract anomalies were demonstrated by urography in 31 patients, eleven boys and 20 girls. In 22 of these cases, 11 girls and 11 boys, obstruction of the urinary tract was present.

Bacteriological examination of the urine was undertaken before, and again two days after, termination of treatment. In girls, all the samples were obtained by catheterization, in boys clean voided midstream specimens were used. The pretreatment examination showed growth of bacteria in 146 cultures altogether. The distribution of the different species was: *E. coli* 56%, coliform rods 8%, *Proteus vulgaris* 17%, Enterococci 5%, other species (*Proteus Morgani*, *Aerobacter aerogenes*, *Staphylococcus pyogenes*) 4%, and mixed flora 10%. The most common combinations of species found in the mixed flora was: *E. coli* and *Proteus vulgaris*, and *E. coli* and Enterococci.

The post-treatment cultures were positive for bacteria in 44 cases. In 19 cases the pretreatment flora was totally eradicated and replaced by new species, in 10 cases new species were added to the pretreatment flora, and in fifteen cases this flora persisted unchanged. It is of course possible that changing of the flora has in reality been still more common. In an apparently persisting infection caused for example by *E. coli*, a new strain can replace the original without this being discovered by ordinary bacteriological examination. The distribution of the different species in the post-treatment flora was: *Proteus vulgaris* 23%, *E. coli* 18%, Enterococci 11%, *Staphylococcus pyogenes* 9%, *Pseudomonas aeruginosa* 5%, *Proteus Morgani* 2%, mixed flora 32%. The post-treatment flora is characterized by a more common occurrence of such species as *Proteus*, Enterococcus, *Staphylococcus pyogenes* and *Pseudomonas*.

Resistance determinations in vitro were done by a paper disc method. Most strains were tested according to the technique described by Ericsson, Høgman and Wickman (1954). As it was considered that bacteria belonging to sensitivity groups I and II would probably be accessible to therapy, these strains will in the following be termed sensitive. Strains belonging to sensitivity groups III and IV will be called resistant. The resistance determinations were all carried out in The Bacteriological Laboratory, Ullevål Hospital.

Altogether 71 strains of *E. coli* were examined. Approximately 80% of these strains were sensitive in vitro to the tetracyclines (Aureomycin, Terramycin, Tetracycline), 90% to chloramphenicol, 70% to sulphathiazole, and only a few strains were resistant to streptomycin and furadantin.

Of 36 strains of *Proteus vulgaris*, approximately 90% were sensitive to streptomycin and chloramphenicol, 60% to sulphathiazole, and 70% to furadantin. All these strains were resistant to the tetracyclines.

Approximately 80% of the 22 Enterococcus strains were sensitive to the tetracyclines and erythromycin, 95% to chloramphenicol and furadantin, 40% to streptomycin, and only a few strains were sensitive to penicillin. This species

is resistant to sulpha-drugs. Only an insignificant number of strains belonging to other species were tested.

It is a significant fact that the post-treatment flora in these infections always has a considerably higher frequency of resistant strains than the pre-treatment flora. Thus of the pretreatment flora in this material (103 strains) approximately thirty per cent of the strains were resistant to the tetracyclines, 7% to streptomycin, 4% to chloramphenicol, 37% to sulphathiazole and 7% to furadantin. In the post-treatment flora (49 strains) approximately 60–70% of the strains were resistant to the tetracyclines, 47% to streptomycin, 33% to chloramphenicol, 57% to sulphathiazole and 37% to furadantin.

The bacteriology of urinary tract infections is often complicated. In 10% of the cases there will be found a mixed pretreatment flora, consisting of two, three, or sometimes even more, different species. The urinary flora may change with each recurrence. Another problem is the changing of the flora during treatment, and the occurrence of a resistant post-treatment flora. In approximately 30% of the cases this flora is further complicated with a mixed flora.

The possibility of contamination of the specimens can never be eliminated with certainty. It is almost impossible to get a normal material for the determination of the frequency of contaminations. In the Childrens Department of Ullevål Hospital, bacteriological examination of the urine was practised on admission in all cases of acute glomerulonephritis. In 113 cases followed very closely over periods of several months without there being signs of urinary tract infection, urine cultures were positive for bacteria in 33 cases (29%). Of 51 girls, the cultures were negative in 40 cases, *E. coli* was found in six cases, *Proteus* in 1, a mixed flora (*Coli* and *Proteus*) in 1, and apathogenic micrococci in 3 cases. Of 62 boys, the cultures were negative in 40 cases, *E. coli* was found in 3 cases, coliform rods in 2, *Proteus vulgaris* in 2, *Enterococci* in 5, and apathogenic micrococci in 10 cases. If one considers the specimens containing only apathogenic bacteria to be negative, the frequency of contaminations in girls (catheter specimens) was 16 per cent, and in boys (clean voided midstream specimens) 19 per cent.

The relatively high incidence of contaminations makes it clear that the occurrence of a positive urine culture is not in itself sufficient for a diagnosis of urinary tract infection. It is now commonly accepted that a determination of the number of bacteria in the urine is of considerable value. If urinary tract infection is present, the number of bacteria is high, in most cases more than 100,000 microbes per ml. urine. Otherwise the number is low, being not more than 5,000 microbes per ml. urine, and often less than 1,000 microbes per ml. In the present study, determinations of the number of bacteria in the urine were not carried out. From a practical point of view, however, much can be gained by a microscopic examination of uncentrifugated urine. In contamination no bacteria will be found.

In the treatment of urinary tract infections, it is of utmost importance that a thorough urologic examination be undertaken in every case of persistent or recurring infection. According to Campbell, chronic urinary tract infection is twenty times more common in children with urinary tract anomalies than in those without. If an obstruction of the urinary tract is present, the therapeutic problem will first of all be surgical. Long-lasting preoperative drug treatment should be avoided, as very often the result will be that a sensitive pretreatment flora is replaced by more resistant secondary species.

In every case of urinary tract infection, there should be carried out a bacteriological examination of the urine with in vitro resistance determination of the isolated bacteria. The value of in vitro determinations of bacterial sensitivity is subject to discussion, and according to certain writers exaggerated value has been placed on these tests. On the other hand, many writers have reported a good parallelism between in vitro bacterial sensitivity and the results of drug therapy.

In the present series, sufficient data for a preliminary estimation of the results of treatment was obtained from 52 patients representing 64 courses of treatment. Patients with obstructive uropathy are not included. In 57 cases with sensitive pre-treatment flora, the post-treatment cultures were negative in 32 cases. Persisting flora was found in 15 cases, a change of flora in 10 cases, and development of resistance during treatment in 12 cases. In 7 cases with resistant pretreatment flora, cultures were all positive after treatment. The results of this small series thus point to a good correlation between the effect of drug therapy and the results of the bacterial sensitivity tests.

During recent years, increasing emphasis has been laid upon chronic and recurring urinary tract infections. Chronic pyelonephritis can take a very long and insidious course, and it has been stated that recurring infections very often represent acute exacerbations in the course of a chronic pyelonephritis. Modern drugs are effective against acute infections, but the therapeutic results in the chronic and recurring cases are still far from satisfactory. The changes of urinary flora during treatment and the development of resistance are very important problems. To prevent such a development, drugs with optimal effect judged by in vitro sensitivity tests should be used in doses which give an adequate serum concentration. After treatment, a thorough bacteriological examination is necessary. A persisting bacteriuria is important, as it may be the only symptom of a persisting, subclinical infection.

Much is still obscure in the pathogenesis of urinary tract infections. In those cases in which obstruction and urinary stasis are not present, very little is known about the reason for the great tendency towards recurrences which is often evidenced. Interstitial fibrosis and scarring with dilation of some tubuli may be the result of an acute pyelonephritis, and these changes in the renal parenchyma may explain a later tendency towards recurrences.

## Nocturnal Enuresis; Aetiologic Aspects

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The average child generally ceases bedwetting during its third year of life, but the time varies greatly from case to case. Not until bedwetting occurs at higher age levels is it referred to as enuresis. Nocturnal enuresis is generally defined as repeated involuntary micturition during sleep after the fourth year of life. Some investigators set the limit as early as three years, others at five. The definition does not include cases of urinary incontinence caused by any gross organic lesion in the urinary tract, the nervous system or by systemic diseases, e.g. ectopic ureters, lesions of the spinal cord, diabetes insipidus. It would be of advantage to distinguish between cases that are secondary to minor organic diseases and mental stress. Such differentiation, however, is not possible, since it is very difficult to evaluate the causal significance of such factors in individual cases.

Nocturnal enuresis is a common symptom during the growing years. Probably about 12% of boys and about 8% of girls in the general population bedwet regularly after the age of four for longer or shorter periods<sup>1, 7</sup>. In the majority of cases, about 75–80%, enuresis is primary, i.e. the children have never had any longer connected dry period before the commencement of enuresis. Enuresis beginning after a dry period of about one year or more, is henceforth called «acquired». In about 1/4 of the cases, bedwetting is combined with daytime wetting, and additionally in a number of cases with diurnal frequency of micturition and urgency. There is a relationship between nocturnal enuresis and encopresis, but this appears to be limited to the cases where bedwetting is combined with daytime wetting or diurnal frequency<sup>9</sup>.

Enuresis is by no means a serious affliction in the sense of being physically invaletudinary, but it is often the cause of serious mental suffering for the affected child and often for its parents. The symptom is the object of great interest in the pediatric, child psychiatric and urological fields, and a very large number of works have been published on its aetiology and therapy. It is true of the majority of enuresis cases that no effective treatment is known. It is obvious that suitable treatment demands a thorough knowledge of aetiology and pathogenesis. Systematic investigation of the genesis of the various forms of enuresis is therefore of great importance.

The nocturnal enuresis symptom is heterogeneous from an aetiological viewpoint, and may occur in conjunction with somatic diseases, mental defects and emotional stress. Genetic factors are additionally of significance for its manifestation. A reliable aetiological analysis of enuresis is therefore highly

complicated. It should be based on representative series divided into clinical sub-categories, and adequate control series of non-enuretics. In the analysis of each separate factor, other factors which may affect the result must also be kept under control. Since only a few investigators have tackled the problem systematically, it is not surprising that opinions are divided as to the aetiological significance of various factors.

In the following outline the various groups of causal factors will be dealt with separately.

#### SOMATIC FACTORS

The significance of somatic disorders as the cause of nocturnal enuresis has been the subject of much discussion. As mentioned previously, bedwetting which is secondary to gross organic lesions is excluded by definition. Such cases are rare. They probably comprise only a small percentage of all children who bedwet after the age of three or four, <sup>9, 17</sup>.

Among the somatic factors, special attention has been paid to defects in the urinary tract. Several investigators who have conducted special examinations of the lower urinary tract in enuretics, state that they have found organic changes of different kinds in about half the cases <sup>2, 4, 7, 12, 14, 31</sup>. The most common disorders are said to be cysto-urethritis, particularly trigono-urethritis and verumontanitis, as well as vulvitis. Stenosis of meatus externa and folds or valves in the urethra are also said to be relatively common. It is not known to what extent these disorders in the urinary tract are of causal significance for enuresis.

It is of interest to note that the changes observed in children with nocturnal enuresis are persistently more common in the cases where this symptom is complicated with diurnal enuresis <sup>17</sup>. In certain cases, an acquired enuresis begins at the same time as an infection of the urinary tract.

Of special interest are the results recently reported by Kjellberg, Ericsson & Rudhe <sup>17</sup> on the basis of comprehensive and thoroughly investigated material. This comprises 598 patients with nocturnal enuresis among 1,155 children aged one day to 15 years who were consecutively referred to their laboratory. The patients were examined with urethro-cystography during micturition by a specially devised method. In the cases where examination showed «severe pathologic» changes, endoscopy was also carried out. The authors could not show any specific organic changes, but they found changes with urethro-cystography, described as severely pathologic, in 21% of the children. In 80% of these cases, pathologic conditions were also observed by endoscopy, in most cases in the form of trigono-urethritis.

Kjellberg and his co-workers also investigated with their method the occur-

rence of urethral valves. They found valves, defined as folds in the posterior urethra, which are a cause of urinary obstruction, in 3.7% of the patients with enuresis.

Some investigators have made the interesting observation that a number of children with nocturnal enuresis discharge greater amounts of urine at night, both absolutely and in relation to discharge during the daytime<sup>23</sup>. In a later investigation carried out under controlled experimental conditions<sup>29</sup>, however, no difference in this respect could be proved between a group of children with nocturnal enuresis and a control group. The material is limited — it comprises 22 enuretics and 24 control subjects — but indicates that nocturnal polyuria is not a common phenomenon in enuretics, if the supply of liquid is kept under control.

Children with enuresis appear on the average to have a lower effective bladder capacity than other children, i.e. they tolerate a smaller volume of liquid in the bladder. The reason for this is not known.

Organic defects in bladder innervation are practically without exception combined with urinary incontinence<sup>17</sup>. The most common cause is congenital myelodysplasia in the form of myelo-meningocele.

Spina bifida occulta is probably of significance as the organic basis of urinary incontinence only if it is combined with myelodysplasia and definite disorders in bladder innervation or other neurologic symptoms in the same region<sup>16, 17</sup>.

The hypothesis prevailing earlier in certain quarters that there is a relationship between nocturnal enuresis and epilepsy, and that enuresis in certain cases is the only symptom of epilepsy representing a nocturnal epileptic attack, has not gained any support from electroencephalographic studies during nocturnal micturition<sup>11</sup> nor from statistical analysis of the frequency of epilepsy among children with and without nocturnal enuresis<sup>8, 9</sup>. In general, it is seldom that neurologic or other organic defects are the direct cause of nocturnal urinary incontinence.

The interpretation of the cause of enuresis from a physiological viewpoint will be discussed later. Here it will suffice to point out that there appears to be a connection between nocturnal enuresis and deep sleep, and that children with nocturnal enuresis to a far greater extent appear to have a dysrhythmic E.E.G. 6, 20, 22, 28.

#### PSYCHIATRIC FACTORS

Systematic studies based on representative series of enuretics and control subjects show that there is a positive relationship between emotional disturbances, behaviour disorders and nervous symptoms on the one hand, and nocturnal enuresis on the other. This connection is strongest in cases where

bedwetting is combined with wetting in the daytime<sup>8, 9</sup>. There is particular interest in the observation that on the average the duration of enuresis is longer in individuals with psychiatric problems than in others<sup>19</sup>.

Nocturnal enuresis is not confined to any particular personality structure, but it has been proved that emotional immaturity is more common among children with nocturnal enuresis than among children not having this symptom.

Low-grade mentally defective children are as a rule incapable of learning vesical control. Thus Thorne<sup>26</sup>, investigating inmates of a state school for mental defectives, found nocturnal enuresis in 84% of «idiots», compared with thirteen per cent among «imbeciles» and 4% among «morons». In general the mental level of enuretic children does not differ to any considerable extent from that of non-enuretic children<sup>8, 13, 15, 25</sup>. Investigations indicating the opposite are probably based on selected material.

It is often very difficult to decide in individual cases whether an existing mental disorder is causally connected with enuresis. The problem is least complicated in acquired enuresis, which not infrequently appears in conjunction with mental disturbances and is, at least in certain cases, certainly a direct consequence of emotional disorders in the child. Acquired enuresis, however, comprises only a minority, probably not more than 20—25% of all enuresis cases.

In primary enuresis the interpretation of the connection is obviously considerably more difficult. The examination of certain social factors (see below) and the analysis of individual cases, however, supports the assumption that even primary nocturnal enuresis is in certain cases the consequence of some kind of emotional stress.

#### ENVIRONMENTAL FACTORS

In the analysis of the aetiologic significance of an environmental factor, a statistical examination of representative subjects should first be conducted to establish whether there exists a connection between this factor and the symptom dealt with, in this case nocturnal enuresis. The nature of the connection is then examined. In certain cases the investigator is referred solely to an analysis of individual cases. Such an investigation may be of value in throwing light upon the problem, but is liable to be subjective and may easily lead to erroneous conclusions.

The analysis of the rôle played by the external environment offers certain difficulties in the nature of principle, which are particularly marked with regard to environmental pressure during the first years of life; and it is the stimulus of the environment during these very years that may be of aetiological significance in primary nocturnal enuresis. First of all these difficulties are

a matter of the criterion adopted. Only certain confirmable environmental data can be defined to any exact degree. Other factors which may be of significance but which cannot be defined in a satisfactory manner do not permit of dependable scientific treatment. Next, certain unfavourable environmental stimuli in the home may ultimately be due to psychiatric disorders in the parents. In such cases it may be difficult to determine whether the nervous symptoms in the children are primarily exogenously provoked. Furthermore, in a number of cases a behaviour aberration in the child is the primary cause of an unfavourable parent attitude. Finally, it should be borne in mind that different children are not all affected so strongly by the same environmental stimuli.

A number of works have been published which illustrate the connection between enuresis and environment. Examination of representative series indicates that nocturnal enuresis is more frequent in the lower social strata <sup>1, 8, 9, 10</sup>.

Acquired enuresis begins in a large number of cases, which in child psychiatric series appears to be in the majority, in connection with some kind of emotional stress, which probably acts as the precipitating agent. The most common factors appear to be the birth of a brother or sister and separation from the mother <sup>9</sup>. It is of interest to recall here the high frequency of enuresis among children who were evacuated during World War II <sup>5, 27</sup>.

A recently published systematic analysis of the occurrence of certain unfavourable environmental factors during the first four years of life in a series of children with primary enuresis and among their unaffected sibs <sup>9</sup> showed that the affected children had more often been subject to the following environmental stimuli: broken home, separation from mother, maternal psychiatric disorder and unfavourable background, defined on the basis of a subjective appraisal of the parent attitude. It is of interest to note that the connection was statistically significant only for the group of affected children where nocturnal enuresis was combined with diurnal enuresis. Further, the »mother-child separation» factor was more common among the solitary cases than among those with a familial occurrence of enuresis. These observations, in conjunction with the observations mentioned earlier that similar environmental stimuli can cause acquired enuresis, indicates that even primary enuresis — at least nocturnal enuresis when combined with diurnal — in a number of cases may manifest itself as the result of unfavourable environmental influences which disturb the emotional security of the child. Individual case histories support this interpretation.

#### GENETIC FACTORS

It is a general observation that nocturnal enuresis often occurs in several members of a family, but opinions are divided on the interpretation of this

phenomenon. A number of investigators are of the opinion that it can be explained by genetic factors, others that the cause is to be found in exogenous factors. In the latter case the explanation of family occurrences is that the various family members are exposed to similar environmental stimuli.

In a current examination of twins, the author has found high concordance as regards nocturnal enuresis among monozygotic twins, while dizygotic partners of twins with nocturnal enuresis do not appear to be affected more frequently than brothers and sisters in general. These observations agree with the hypothesis that genetic factors are at work.

A recently conducted genetic investigation of families<sup>9</sup>, based on 203 propositi with nocturnal enuresis, as well as their brothers and sisters and parents, gave the following results:

In about 70% of the families, enuresis occurred in more than one member of the family, and in over 40% at least one of the parents had suffered from nocturnal enuresis. On the average, about 30% of the fathers and brothers of the propositi and about 20% of the mothers and sisters had also suffered from enuresis. If either of the parents of an affected child was also affected, the morbidity risk among the brothers was about 40% and among the sisters about twentyfive per cent. These figures are considerably higher than corresponding figures — 12 and 8% respectively — for the general population.

The morbidity risk, as expected, was higher among parents and sibs of children with primary enuresis than among parents and sibs of children with acquired enuresis. The latter cases also occurred less often family-wise. Otherwise no definite connection could be shown between the clinical picture of enuresis on the one hand and the familial occurrence of the symptom or morbidity risk among parents and sibs on the other. Neither was there any definite difference, in respect of the morbidity risk among parents and sibs, between children with and without mental disorders or between children with and without unfavourable family backgrounds.

The following observations indicate that the high morbidity risk of nocturnal enuresis among parents and sibs of affected children represents the action of genetic factors:

- (1) A high degree of agreement in the morbidity risk of nocturnal enuresis between parent and sib groups.
- (2) A higher morbidity risk of nocturnal enuresis among brothers and sisters in families with affected parents than in other families.
- (3) A higher morbidity risk of nocturnal enuresis among parents and sibs of children with primary enuresis than among parents and sibs of children with acquired enuresis.

Minor mental aberrations in which genetic factors are of significance for the manifestation cannot *a priori* be assumed to follow simple Mendelian laws.

This is due in part to the very fact that environmental stimuli can modify the manifestation. It has not been possible to establish with certainty any definite mode of inheritance for nocturnal enuresis. The most likely hypothesis, based on empirical data and theoretical evaluation, is that enuresis, in cases where it is caused genetically, either represents the effect of a dominant major gene whose manifestation is modified by environment and polygenes (i.e. genes with additive effect) or is determined solely by the combined effect of environment and polygenes.

#### DISCUSSION

It is justifiable on the basis of current investigations to draw certain conclusions about the aetiology of nocturnal enuresis. The symptom is heterogeneous from an aetiological viewpoint. It can be the consequence of a disorder of the urinary tract, but this appears to apply only to a minority of the cases. It is probably somewhat more common that enuresis is a non-specific neurotic symptom, which can be precipitated by emotional stress. There is particular reason to search for exogenic factors as the cause when enuresis is acquired, and when nocturnal enuresis is combined with diurnal enuresis. There is, with a high degree of probability, a «nuclear group» of cases where enuresis is primarily genetically determined. Even in this group, which probably includes the majority of cases, the manifestation is modified by exogenic factors.

Our knowledge of the physiology and consequently also of the pathogenesis of enuresis is still incomplete. Physiologic examinations of micturition<sup>3, 18</sup> show that the cerebral control of reflex bladder evacuation is inhibitory in nature. Cystometric studies of enuretics<sup>21, 24</sup> appear to indicate that this inhibitory control may be impaired in patients with nocturnal enuresis.

Even other observations already referred to, such as the high frequency of children sleeping deeply and with a dysrhythmic E.E.G. among enuretics, and also possibly the commonness of certain mental immaturity symptoms, indicate that enuresis in a number of the cases is combined with some disturbance of the cerebral functions.

If the results of the investigations described prove to be tenable, it is reasonable to assume that such a lack of inhibitory control over the bladder could be the symptom of a neurotic reaction as well as of cerebral immaturity. The latter may well be genetically determined.

In his review of nocturnal enuresis, in children, Walshe<sup>30</sup> maintains, that the hypothalamus plays an essential part in the regulation of sleep and in the liberation of the so-called anti-diuretic hormone in the posterior lobe of the pituitary, and that this hormone is considered to be inactive during deep sleep. Whether these phenomena have any pathogenic significance for nocturnal enuresis has

not been established. As has already been pointed out, the published results of investigations are not unanimous with regard to the occurrence of nocturnal polyuria in nocturnal enuresis.

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### Arterial Hypertension in Unilateral Kidney Disease

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Three cases are discussed of severe arterial hypertension in childhood caused by unilateral kidney disease. In two of the patients, there was chronic pyelonephritis with secondary atrophy of one kidney, and in the third patient a narrow segment and also an aneurysma on the renal artery could be demonstrated by angiography. Following nephrectomy, two of the patients became normotensive, while the third patient required in addition hypotensive medication (reserpine) to get normal blood pressure.

The importance is stressed of performing intravenous pyelography and, if necessary, renal arteriography in all cases of obscure arterial hypertension in childhood. The prognosis following adequate treatment (surgical and, in some cases, medical) is better than in adult patients with hypertension due to unilateral kidney disease, but not uniformly favourable, about two thirds becoming normotensive in large series.

## Cystic Kidneys in Newborns, Infants and Children

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By means of morphological and clinical studies of cystic kidneys from newborns, infants and children, we have been able to classify the malformations according to their histological appearance into 5 groups, of which two — groups I and II — are distinct entities.

Group I comprises kidneys from 9 children in 6 families. Being enormously enlarged, these kidneys exhibit a gross spongy appearance and histologically the parenchyma, with the exception of a tiny narrow zone next to the cortex containing scattered and in part rudimentary glomeruli, is entirely occupied by small, round or elongated cystic spaces. Fully normal nephrons are never seen. Both the microscopical appearance and the clinical course (e.g. the existence of dry labour) suggest that these kidneys cannot function. Clinically these children present, in addition to the distended abdomen, the so-called Potter's face, and they expire within a few hours of birth, probably of respiratory insufficiency. Cysts in the pancreas and/or liver were found concomitantly with the renal malformation in half these cases. The nature of the malformation and its familial incidence constitute strong evidence of a recessive heredity.

The risk for a family with this recessive gene of having an abnormal child is accordingly one in four. The prognosis for the affected child is exceedingly pessima. For siblings who do not exhibit the clinical symptoms described, and who do survive the first day, the risk of their having cystic kidneys is nil, but, in accordance with the above assumption, two thirds of the healthy children will carry the gene.

In practice, this implies that when a case of cystic kidneys of group I has occurred in a family, the prognosis for subsequent children is reasonably favourable, and so the parents, provided they are aware of and understand the risks involved, need not be cautioned against having any more children. It should be noted however, that the healthy children should be strongly discouraged from contracting consanguinous marriages.

As regards group II, comprising 10 children in 7 families, the prognosis is completely different. These children are also born with very large kidneys, which often cannot be distinguished from the kidneys in group I other than by the microscopical appearance with areas of intact parenchyma and functioning nephrons. These children may survive the neonatal period, and for some time exhibit no symptoms of their renal lesions. Here also the deformity occurs in families, and there is much to suggest that it might be a condition of the same kind as the adult, dominantly inherited type of cystic kidney. Although

we have not been able to prove this thesis, the fact that the children frequently are viable and go on living for quite a time constitutes a sufficient reason for strongly discouraging a couple from having additional children if they have had a child with kidneys belonging to this group. In our opinion a legal abortion is strongly indicated if a mother in such a family again becomes pregnant.

The other groups were not characterized by any clinical or pathoanatomical peculiarities.

Our investigation has shown that the histological relationships must be carefully charted before a proper clinical evaluation of a case of cystic kidneys can be made. A simple diagnosis such as «typical cystic kidneys» is in the individual case meaningless, and cannot be made the basis of a prognosis for subsequent siblings.

### **Renal Concentration Capacity during Acute, Nonobstructive Urinary Tract Infections in Infancy and Early Childhood**

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Six cases have been examined after subsidence of the acute symptoms. The concentration capacity was found to be lowered in 5. The findings suggest a damage of structures of the nephron responsible for the final adjustment of urine concentration, but whether this damage was localized mainly to distal tubules, collecting ducts, or to both, cannot be elucidated from the present investigation. It seems probable that such impairment is often present even in the mild cases of urinary tract infections in infancy, often classified as cysto-pyelitis.

The findings are reported in detail in *Acta paediat.* 47:635, 1958 and *Acta paediat.* 48:1959.

### **On the Prognosis of Urinary Tract Infection in Childhood**

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Helsinki, Finland

During the ten-year period 1946—1955, 234 cases of urinary tract infection were treated in the Children's Clinic. The findings in urological examination were as follows:

Urological examination	females	males
not done .....	82	12
normal finding .....	37	9
pathological finding .....	60	34
lower urin.obstr. ....	16	16
hydronephros .....	13	5
ureterocele .....	11	1
other anomalies .....	20	12
total .....	179	55

On the basis of autopsy records, follow-up inquiries and follow-up examinations, information was gained about 148 females and 41 males:

Results	females	males
symptom-free .....	88	20
recurrent urinary infections .....	44	7
elevated blood pressure .....	15	6
deaths .....	8	10

**Conclusions:** A childhood urinary tract infection is accompanied by a considerable morbidity: recurrent urinary tract infections in approximately 30 per cent of females and 20 per cent of males, elevated blood pressure in about ten per cent in both. Mortality was approximately 6 per cent in females and twentyfive per cent in males. In a high percentage of cases, the morbidity and mortality were due to an underlying abnormality of the urinary tract. As the symptoms of urinary tract infection, especially in infancy, are vague, the catheter urine specimen should be examined in all suspected cases before any antibiotic treatment or chemotherapy is given. The efficacy of treatment should be checked by repeated catheter urine examinations. In all recurrent cases, a thorough urological examination should be made.

#### DISCUSSION

*P. W. Bræstrup*, Denmark. — At Gentofte hospital, the general principles for examination and treatment seem to be a little more conservative. According to a review to be published shortly in *Ugeskrift for Læger* by Dr. J. Lund for instance 7 out of 12 cases of reflux (all girls with recurrent pyuria) were treated successfully by frequent miction alone or triple miction (acc. to Stephens); four were operated on (ureteroplastic a.m. Hutch mod.) and all cured. Of twelve cases of hydronephrosis (4 girls and 8 boys), only one had a nephrectomy in our hospital and one in another; the rest were successfully treated by con-

servative plastic corrections. Of 5 cases of ureterocele, 2 could be successfully corrected, 3 (all in ectopic ureters) had hemi-uretero-nephrectomy performed.

The necessity of conservative and expectant treatment of reflux in the ureters is underlined by Dr. Lund's finding of this symptom in 4 out of 64 completely normal children with no complaints whatsoever.

### **Nocturnal Bedwetting; An Attempt to Treat School Children with Banthine and Pro-Banthine**

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This paper gives an account of an attempt to treat nocturnal bedwetting in school-children without regard to the aetiology of the disorder, as a number of papers have been published with the conclusion that a treatment such as Banthine or Pro-Banthine given late in the evening would make it impossible for the children to empty the bladder during the night<sup>1, 2, 4</sup>. If this were true, it would be so much easier to search for the aetiology while the patient was kept dry.

There is no intention of discussing the possibilities of aetiology, the trouble this ailment can produce in a family, or all the various forms of treatment which have been employed.

The case material consisted of 95 children from 2 private and one public schools. In the schools, altogether 2,542 pupils (1,417 boys and 1,125 girls) belonged to the lower and middle grades (the first 9 years), and of 54 boys and fortyone girls, it appeared from their health charts that they suffered from nocturnal enuresis.

After the co-operation of their homes and the permission of the family doctors had been obtained (because the school physician in Denmark is not allowed to give any kind of treatment) the numbers of wet nights without medicine were counted over a period of 5—6 weeks. Thereafter 26 children (12 boys and 14 girls) were not taken into further consideration because they were wet so seldom that it would be impossible to tell of any effect if the drugs concerned should happen to be active.

Of the remaining 69 children, 42 were boys, and 37 girls. Of these, 23 had six to seven wet nights a week, 17 had 4—5 and 29 had 2—3 wet nights every week. The material was divided into 3 unselected groups which were given three different kinds of tablets according to the double blind method. In group 1, a child started with having tablet No. 1 and was told to have one

tablet each evening. If it was found effective, the medication went on for six weeks and every dry night was noted on a calendar. If it was not effective after 3 weeks, 2 tablets of No. 1 were given in the evening. After 6 weeks, No. 2 was tried in the same manner, and after another 6 weeks No. 3 tablet was given. Group 2 started with tablet No. 2 and so on.

Unfortunately the experiment could not be completed with all the children because some of them refused to take the tablets as a result of undesired complications, some went away to hospital or recreation camp or moved away. Finally, only 47 children went through the whole test, but in the statistical analysis all the results which could be used have been taken into consideration.

The age and the grades of the children appear in table 1:

Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Age	7		8		9		10		11		12		13—15			
	8	10	13	6	6	3	2	2	9	3	2	2	2	1		
In all	18		19		9		4		12		4		3			
School year	1		2		3		4		5		6		7			
	9	10	12	7	5	1	7	3	5	3	2	1	2	2		
In all	19		19		6		10		8		3		4			

The complications were noted in 21 patients and are shown in table 2:

Tablet	Number	Bad taste or difficulty in swallowing	Gastro-intestinal symptoms	Dryness in mouth and throat	Exanthema	Excessive sweat	Aggravation
No. 1	7	1	2	2		1	1
No. 2	4	1					3
No. 3	15	7	5	2	2		1

During the period of this clinical experiment, which lasted nearly half a year (24 weeks), a close contact with the patients and the homes was maintained, mainly by the two health nurses Miss Dorph-Petersen and Miss Refslund Poulsen, who were excellent assistants, and at the end the parents were asked to fill in a questionnaire. From the replies, it appeared that in 42 cases some effect had been obtained, and for most of the children the result was found to be better with one of the tablets only, but there was striking disagreement about which tablet was the best one. In 20 children, No. 1, which was Pro-Banthine, was said to be effective, in 8 cases No. 2, which was a placebo, gave the best result and finally in 17 patients No. 3, which was Banthine, was efficacious. All the mothers had been given calendars on which they marked the given dose each evening, and a cross in the morning if the bed was dry. From the appearance of the calendars, it was sometimes difficult to understand

why the parents had found a tablet beneficial, but it must be admitted, however, that in some children a definite improvement was obtained, and that could happen with any of the tablets.

It was striking, though, to see that without regard to which tablet was given first or last, the improvement generally appeared in the final period of the experiment. Thus the effect was claimed by the mothers to occur in the first, second and third period in 6, 13 and 23 cases respectively. It was therefore very fortunate that the children had been divided into 3 groups which started and ended with different tablets. Otherwise the effect of the last tablet might have been interpreted as proved in comparison with that of the other tablets, and this would have been unjustified.

An elaborate statistical analysis has been carried out by Gudmund Rasch, Ph. D. and Arne Nielsen, actuary. At first they made an examination to determine if it made any difference whether one or two tablets were given, and it was shown that no significant difference could be found either with boys or with girls. It was also found that irrespective of which tablet was given, there were fewer wet nights at the end of the period of the experiment. This was in May, when the nights are shorter, and it was also found that the placebo tablets were less effective than the drugs if the children in the test period were taken out of bed to urinate during the night, especially as far as the girls were concerned. Whether this means that the effect would be more pronounced if the drug tablets were manufactured so that they were more gradually absorbed, and thus worked during a longer period, has not been elucidated because the manufacturer (Searle, who kindly put the tablets at my disposal through their Danish agent Ercopharm) has not found it worth while to try this alteration.

The conclusion was the disappointing one that no significant effect ascribable to the drug was obtained with any of the tablets (as Mayon-White<sup>3</sup> also found), when one or two tablets were given before bed-time and no other precautions were taken.

*Summary:* Of 95 school children with nocturnal enuresis 54 were boys and fortyone girls. After the co-operation of the parents and the permission of the family doctors had been obtained, the children were given charts on which they put crosses for each dry night during about 6 weeks. 26 of the children had so few wet nights that they were removed from the experiment. The remaining 69 children, 42 boys and 27 girls, were divided into 3 unselected groups. Each group started with different tablets according to the double blind method, and after six weeks the next tablet was tried. Thus all the children had Banthine, Pro-Banthine and a placebo. After a very careful statistical survey, the result appeared to be that no significant effect ascribable to the drug was obtained under the given conditions and with a dosage of one or two tablets at bedtime.

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## DISCUSSION

A. Biering, Denmark. — We have been taught to regard the phenomenon of «enuresis nocturna» as a disease. Dr. Hallgren uses such words as etiology, pathogenesis, disease sui generis, morbidity risk etc., words that classify bedwetting as a pathological condition. According to the definition, nocturnal enuresis is present when a child wets its bed after the age of four. This definition cannot be understood literally, as this would mean that a lot of normal children would be taken ill on their four years' birthday. But how can it be understood?

Different children acquire different abilities at very different ages. The capacity to stay dry at night is subject to a particularly wide variation. Some children get dry at the age of one year; others continue to wet their beds until adult age. What entitles us to postulate that it is not quite as normal for one child to get dry at ten years of age, as for another child to stop bedwetting at the age of one? At any age a certain number of children are bedwetters. Their distribution according to age will probably follow a curve, resembling half of a normal distribution (Crosby: M. J. Australia 1950, p. 533, fig. 1). The actual number at a certain age is difficult to estimate. In a public school in Copenhagen, I found 16 children with nocturnal enuresis out of a total of 131 pupils in the first classes (aged 7 years), viz. 12%. 12 had primary and four secondary enuresis. The study will be continued for some years to get a more precise estimate; but the order of magnitude is probably correct. It is unreasonable to maintain that all these children are ill.

Enuresis should not be regarded as a disease, but as a social nuisance which is due to our civilized way of life, a normal variant, probably a late maturation. Several facts are in accordance with this point of view:

- 1) The phenomenon itself,
- 2) The emotional immaturity which is prevalent among enuretics,
- 3) The electro-encephalographic findings which indicate a cerebral immaturity (Gunnarson & Melin: *Acta paediat.* 40:496, 1951),
- 4) The familial disposition. It is quite natural that the children are of the same type as their parents.

5) The influence of the environment upon the manifestation. The progression of the maturing process is liable to be influenced by the surroundings, which can also cause regression.

6) Finally, I shall once more call your attention to the age curve which makes the establishment of a limit between the normal and the pathologic quite arbitrary.

This point of view will cause a much more tolerant attitude towards enuresis. Much will be gained if we succeed in communicating this tolerance to the parents. I agree with Klackenbergh (*Acta paediat.* 44:513, 1955) who writes: »It ought to reduce refractoriness and aggressions and eliminate many of the problems which civilized man, on account of his foolish zeal, has created around a biological function that takes its natural course.» To return to the starting-point, I prefer not to talk about etiology, because there is no disease and consequently no etiology.

*R. Rinvik, Norway.* — During the years 1951—1957, 186 patients in all (119 boys — 67 girls) were admitted to the Children's Department, Ullevål Hospital, Oslo, for enuresis. It seems to be a hereditary disposition, as more than half of the patients (54%) know about others in the family having wet their beds. The social economic condition does not seem to be of importance, as the patients come about equally from the different classes of the population. About one fifth showed slight psychological deviations, mostly in their homes. Only 6.45% were mentally retarded.

Eeg-examinations were carried out on 159 patients, of which 40 or 25% showed changes of dysrhythmia etc.

A follow-up of 122 of these patients, of whom 107 had been Eeg-examined, showed that the frequencies of continued enuresis were about equal in patients with normal and abnormal Eeg-s.

Of the total 186 patients, 158 were X-ray-examined (urography) and 9 (5.7%) showed anomalies in the urinary tract (most frequently doubled ureter).

*Conclusions:* Enuresis is a manifestation of a developmental anomaly. Psychogenic factors are probably secondary.

*P. Plum, Denmark.* — The frequency of enuresis was examined by asking medical students to tell in writing whether they had enuresis nocturna at the age of 7 years. This questioning of the students twice gave the result as 5 per cent. In two personal examinations of the mothers of children entering school school doctors have found the same figure. As regards treatment, it has become common to believe that it must be psychotherapeutic. I believe that we will often waste time in trying psychotherapeutic measures in these cases, and think that the psychological treatment is an exercise of the reflex mechanism controlling urination.

*P. W. Bræstrup*, Denmark. — With relation to the discussion at this meeting, Dr. G. Buchmann studied 167 consecutive cases treated at Gentofte Hospital during 1955—56. Details will be published in *Nordisk Medicin*.

We find mental problems around the child in a great majority of the cases, for instance 95 cases of «parent problems», but this does not seem to differ significantly from the situation in a group of normal children carefully studied by a staff team. However, school problems seem to be more common in enuretic children, and serious problems were recorded in 14 out of 80 school children. A further 32 of the whole group applied their intelligence poorly, and in all, attempts to correct conditions connected with the school were part of the treatment in 33 cases.

In our cases somatic diseases exceed the average. 33 had adenoid vegetations and sinusitis, and a further 13 had recurrent respiratory infections. A variety of pathologic conditions, including epilepsy (4), hemicrania, constipation etc., were observed in 29 children. All children had a thorough somatic examination; a special family history and psychologic testing of the children was undertaken by a psychologist.

The treatment consisted in the correction of somatic disorders, counselling the family and often in temporary placement of the child in a new milieu.

The results are in accordance with a majority of publications. After a minimum of 1 year of observation 75% were completely «dry» or definitely improved.

For further details see: G. Buchmann, *Nord. med.* 60:1493, 1958.

*B. Hallgren*, Sweden. — Whether nocturnal enuresis is a «normal variant» or a «pathologic condition» is largely a question of definition. I have preferred to call nocturnal enuresis a «disorder» and a «symptom». «Morbidity risk» is a technical term which refers to the risk of an individual to manifest a certain characteristic.

Every characteristic, even if it cannot reasonably be described as a «disease», naturally has an aetiology. Moreover, it represents the combined effect of genetic and non-genetic factors.

Bedwetting is, as regards the age at cessation, a quantitatively distributed variable. There is, however, an accumulation of cases that achieve vesical control on reaching puberty, indicating a discontinuity in the distribution.

From the genetic point of view, quantitatively distributed variables are presumably multifactorially inherited, i.e. they are due to the combined action of several genes with an additive effect (polygenes). The total variation will be the result of the interplay between genes and environment. Nocturnal enuresis may thus be looked upon as a «physiological» variant analogous with mental retardation, for instance.

However, it is reasonable, from the clinical aspect, to discount enuresis

lasting up to the age of adolescence or longer as a «pathological» variant, and, from the genetic aspect, it is probable — also in analogy with mental retardation — that there are cases of enuresis inherited through major genes, i.e. it may be regarded as a disorder «sui generis». If this is true, the manifestation of the major gene would still be modified by polygenes. Accordingly, there should be a not inconsiderable overlap between «physiological» and «pathological» enuresis.

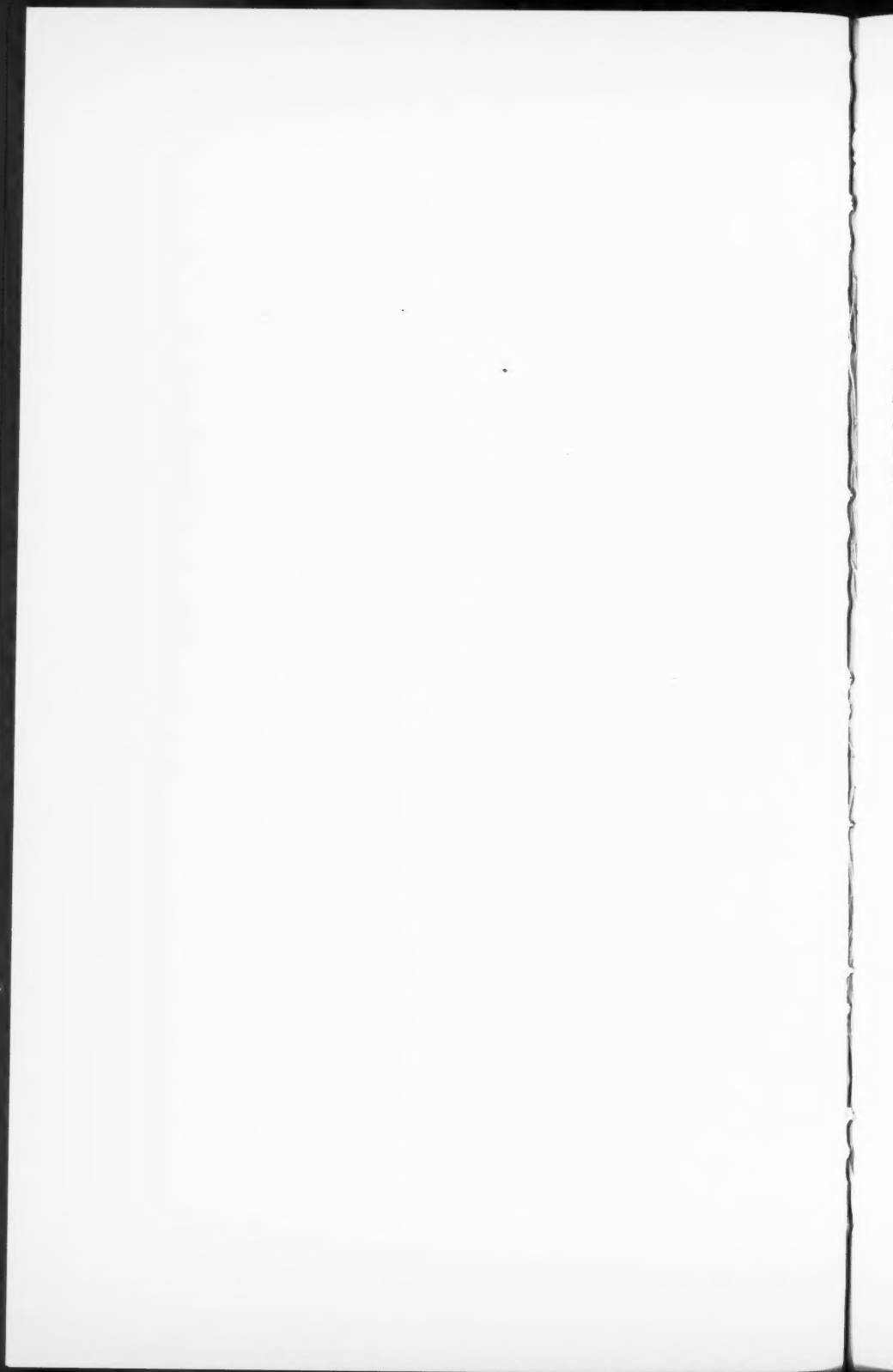
*P. Plum, Denmark.* — I agree with Docent Hallgren that psychological factors are important in some cases. I believe that this is more often the case in secondary enuresis.

*J. Winberg, Sweden.* —

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## SESSION IV



## A Field Study with Live Attenuated Polio-Vaccine

S. GARD, M. BÖTTIGER and R. LAGERCRANTZ

Stockholm, Sweden

An attenuated but cytopathogenic polio-virus Type I (Type »Chat«, Koprowsky) was used in a field-study. Nineteen families participated each with an index-child born in 1956 or later, and with at least one older child. Fifteen of the nineteen index-children, eighteen of thirty siblings and four of forty adults had no antibodies against any polio-virus before vaccination with Swedish formalized virus-vaccine. After two or three injections of this vaccine, all had antibodies against all three types of polio-virus. The index-children were vaccinated perorally with 1 million cytopathogenic units. All became viruscarriers and excreted virus during periods of one to more than eleven weeks. No ill effects were noted. There was a spread of the virus to ten siblings, but not to any of the parents (of whom only four were triple-negative prior to the vaccination with dead vaccine). All index-children and infected siblings showed an antibody-response to polio-virus Type I two—three months after the vaccination with the live vaccine.

The faeces-strain of virus will be studied and compared in detail with the vaccine-strain, to see if there has been a change in the pathogenicity. The index-children and the rest of the families will be revaccinated with live attenuated vaccine to see if they are locally immune and the level of their antibodies will be followed up.

### DISCUSSION

*L. Philipson*, Sweden. — A similar investigation was carried out in Uppsala, Sweden, in a nursery-institution, and there was obtained definite evidence of a higher spread of the live virus vaccine than that reported by Gard et al. Further, there is some evidence in published works that an increase in virulence occurs by several passages in primates, i. e. monkeys, which may entail that the live vaccine only should be restricted at the present time to an immunized population, which has been immunized by a formalin-inactivated vaccine in advance of the live virus vaccine.

## Immune Globulin Against Vaccinia

ROLF LUNDSTRÖM and ÅKE ESPMARK  
Stockholm, Sweden

Passive immunization in connection with virus-induced diseases is probably of most significance in the prophylactic field. This has been perhaps best noted in e.g. measles. It is fairly generally held that little therapeutical help is to be expected through passive immunization in already manifest viral conditions.

Kempe and his associates have, however, succeeded in influencing favourably a case of progressive gangrenous vaccinia, an otherwise fatal vaccination complication which, fortunately, is extremely rare. They employed a gamma globulin prepared by Kempe and obtained from the blood of persons who had been successfully vaccinated recently. The patient, a boy of 4, recovered. Kempe then reported a series of cases treated with this immune globulin, successfully as far as can be judged.

Preparation of an immune globulin of this type was started in 1955 at the State Bacteriological Laboratory. It was made from the sera of conscripts who had been successfully vaccinated against smallpox on entering military service. The blood was drawn c. 4–8 weeks after the vaccination of the donors. Vesicle formation was regarded as a positive vaccination result. The serum fractionation was performed by AB Kabi, Stockholm.

Several cases of complications caused by vaccination against smallpox were treated with this immune globulin. A survey of these cases and a summary of the indications and dosage was reported.

Rapid intervention may lead to a successful outcome in vaccination complications. To prepare for such situations, which are fortunately infrequent, it may be advisable to set up at strategic points supplies for the treatment of one or two such cases. The supplies can then be rapidly distributed when indications for the use of immune globulin are present.

According to preliminary examinations at the State Bacteriological Laboratory, the proportion of antibodies against vaccinia virus is c. 10 times greater in this immune globulin than in the ordinary gamma globulin available in Sweden, extracted from retroplacental blood.

### DISCUSSION

*T. Salmi*, Finland. — Three weeks ago, in the Children's Clinic, Turku, a girl of 1 ½ was being treated for vaccinia generalisata received from her brother, vaccinated a week earlier. The patient herself had an itching eczema which had aggravated the infection. The temperature curve was septic, there was extensive

lymphadenitis. The girl recovered without any specific treatment. A similar case was reported twenty years ago from the Children's Clinic in Helsinki: a child with infantile eczema had caught a vaccinia infection and died. Care must be exercised in inoculating eczematous children and members of a family in which one has eczema.

## **Virologic Aspects on the Upper Respiratory Infections in Childhood with Special Reference to Non-Diphtheritic Croup**

LENNART PHILIPSON

Uppsala, Sweden

During recent years, much interest from a virological point of view has been devoted to the upper respiratory infections. New viruses, such as adenoviruses, croup-associated virus (CA-virus) and some common cold associated viruses have been isolated in tissue cultures. The aetiological connexion between these viruses and upper respiratory disease has yet not been verified, but several reports have appeared which show a definite relation between some types of adenovirus and febrile upper respiratory infections.

In an investigation of the aetiology of non-diphtheritic croup in children, there was isolated a virus, probably belonging to the Echo 11. This has been called Uppsala-virus (U-virus). This virus was isolated in 30 per cent of non-diphtheritic croup children, and serological evidence was received that 40 per cent of the children investigated had gone through an infection with this virus. Further, some evidence was adduced in transmission experiments in adults that there existed a relation between U-virus and non-febrile respiratory disease. A separate virologic study of an epidemic of upper respiratory infection in a Day-Nursery showed that infection with U-virus had occurred in the children during the period of the epidemic. During this epidemic, 4 out of 24 children became ill with characteristic croup manifestations. Earlier investigations showed a probable non-infectious factor to be involved in the pathogenesis of this disease apart from the generally accepted infectious one. It has further been shown that a primary bacterial aetiology seems unlikely. As regards these results, it seems justifiable to assume that the pathogenesis of non-diphtheritic croup may involve an allergic moment, where common respiratory viruses may be the sensitizing agent.

### DISCUSSION

*R. Lundström, Sweden. —*

*R. Lagercrantz*, Sweden. — These interesting investigations have proved that bacteria play a subordinate role in the etiology and course of pseudocroup. However, it must be pointed out in this connection that malignant tracheo-bronchitis is not always distinguished clearly from pseudocroup. Tracheo-bronchitis is in fact a typical infectious disease the etiology of which is often *Hemophilus influenzae*.

This infection should be treated with chloromycetin. But for ordinary pseudocroup it should generally be possible to manage without chemotherapy.

*L. Philipson*, Sweden. — No trial of the antibody content of human  $\gamma$ -globulin has yet been carried out. A separation of the acute laryngotracheo-bronchitis from other types of subglottic non-diphtheritic croup seems highly indicated, but no true correlation between *H. infl.* type B and acute laryngotracheo-bronchitis seems to have been proved to exist. In spite of this, in my view indications for antibiotic therapy still exist in the non-diphtheritic croup syndrome because of the rapid development of bacterial complications in some cases.

### **A Rediscovered Etiological Factor in Idiopathic Hypoglycaemia in Children**

O. BROBERGER, I. JUNGNER and R. ZETTERSTRÖM  
Stockholm, Sweden

Two cases of idiopathic hypoglycemia in childhood with failure to increase the adrenalin secretion in insulin induced hypoglycemia are reported. The possibility of hypofunction of the adrenal medulla as the cause of the hypoglycemic attacks is discussed. This theory is supported by the fact that both children have been completely free from symptoms after continuous ephedrin medication. To test the validity of the theory, one child with hyperinsulinism due to hyperplasia of the beta-cells of the pancreas, and 5 normal children of various ages are investigated according to their excretion of adrenalin after injection of insulin.

#### **DISCUSSION**

*R. Rinvik*, Norway. — The etiology of hypoglycemia is certainly different. Let me report a case: a boy of 4 was hospitalised in a soporific condition, in shock, with blood sugar at 30 mg%. About 1½ days previously he had eaten tiger lily seeds. A similar condition is known in Jamaica as »vomiting sickness».

it arises in the children when they eat a certain fruit at a certain phase of ripeness. In our patient insulin activity was definitely increased 2 days after hospitalization. 3 days later he was fully normal.

Z. Eriksson-Lihr, Finland. —

## The Adrenocortical Function in Children

### Preliminary Report

TOIVO SALMI, AIMO PEKKARINEN and SAARA HEIKKILÄ

Turku, Finland

The adrenocortical reserve and the 17-hydroxycorticosteroid excretion in children of different age groups have been comparatively little studied. It is well known that the hormones are of great importance for the regulation of the growth, development and the metabolism of the child.

#### MATERIAL AND METHOD

We have studied the basal excretion of total 17-hydroxycorticosteroids in one hundred fortyone children from infants to 15 years of age and the adrenocortical reserve during a 2 days' ACTH-test (Acton prolongatum, Nordiska Hormonlaboratoriet, Malmö) in 63 children. We have determined the excretion of total 17-hydroxycorticosteroids in urine after injection of 0.55 units of depot-ACTH per kilogram of body weight, twice daily during two consecutive days. The total 17-hydroxycorticosteroids were determined in the diluted urine by a modification of the method of JENKINS *et al.* (1955) (fig. 1, 2, 3, 4, 5). The accuracy of the 17-OHCS method determined in recovery experiments with a hydrocortisone standard (20 mg/ml) (Ciba, Leiras, Schering) was 92.3 per cent. A standard deviation of two independent duplicates in per cent are seen in table 1.

TABLE 1

DETERMINATION OF 17-HYDROXYCORTICOSTEROIDS IN THE URINE

Mg per 1000 ml	SD. per cent	Number of analyses
2.4	30.0	354
6.8	16.8	326
12.0	11.6	243
22.6	10.8	404
36.8	5.4	170
52.4	3.9	70
68.6	2.8	58

## RESULTS

## 1. Basal excretion

From the first days after birth, the child excretes certain hormones relatively more than two weeks later (SALMI *et al.*, 1957). They are necessary for its adaptation from intrauterine to extrauterine life. They originate partly from the mother.

EXCRETION OF 17-HYDROXYCORTICOSTEROIDS  
MG PER 24 HOURS

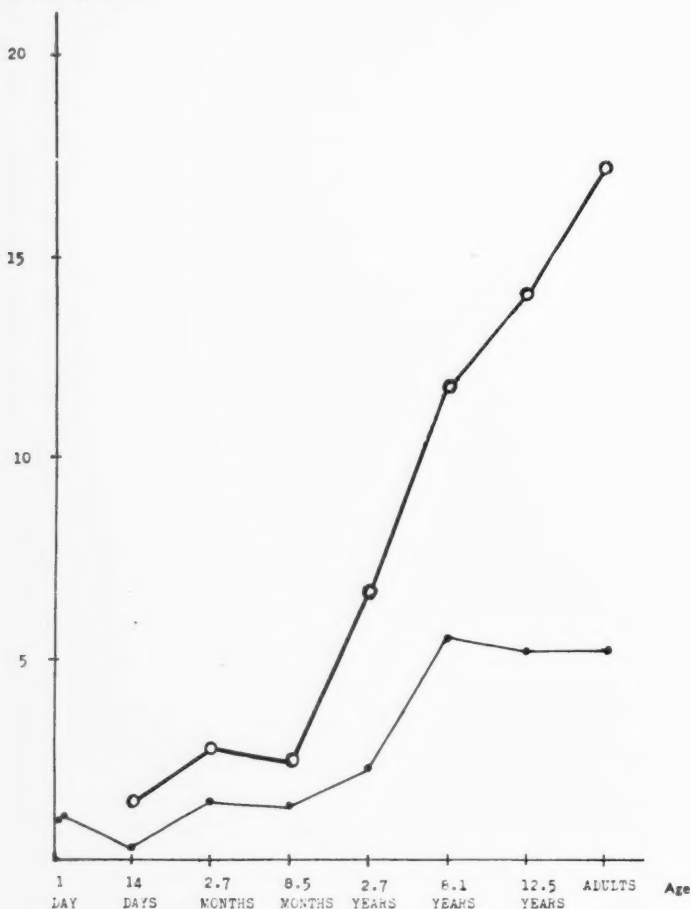


Fig. 1. The excretion of total 17-hydroxycorticosteroids into the urine (mg per 24 hours) in different age groups of children compared with the excretion in adults.

The basal excretion of 17-OHCS = - - - - -

The average excretion of 17-OHCS during the first and second ACTH-days = o—o—o—o—o

During the first and second days of life 1.0—1.1 mg of total 17-hydroxycorticosteroids are excreted (figure 1). During the two first weeks, it is 0.3 mg per day, or less than immediately after birth. However, the basal excretion diminishes gradually during the first month.

The excretion increases again during the first year. From the 6th—12th month it is 1.32 mg per day, or four times as great as in the two weeks old children.

The average basal excretion in the 1—6 year (average 2.7 years) old children still increases. At the age of 4—6 years it is 3.7 mg, or about 3 times greater than at the age of 1—4 years (figure 2).

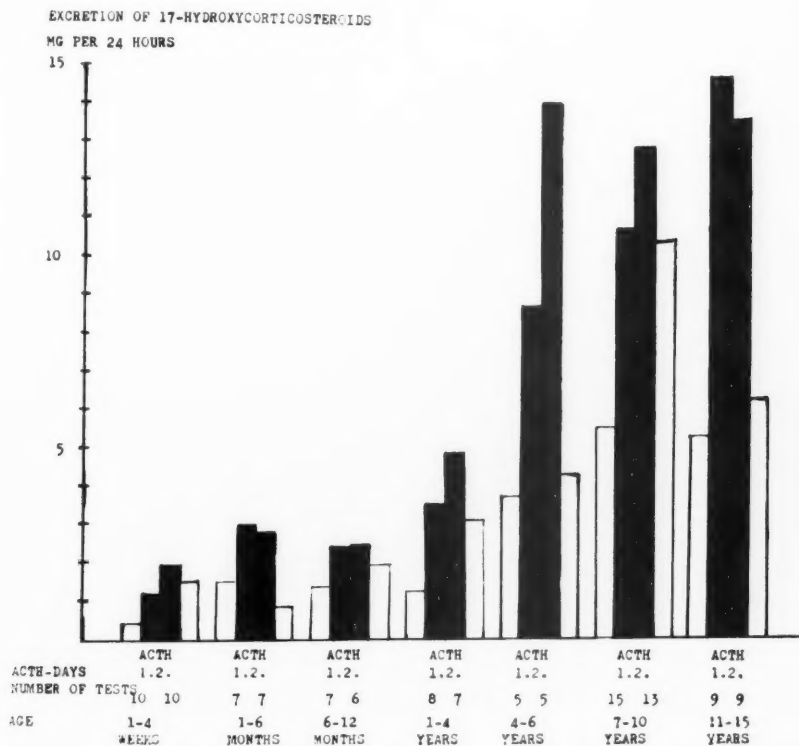


Fig. 2. The excretion of total 17-hydroxycorticosteroids into the urine (mg per 24 hour) in different age groups of children as compared with the excretion in adults.

The basal excretion = the light columns on the left side of each age group.

The excretion during the first and second depot-ACTH-days = the dark columns in the middle.

The basal excretion immediately after the depot-ACTH-test = the light columns on the right.

In the 7–10 year old children, the basal excretion increases continuously and can even then reach the level of adults, 5 mg. When one considers that the child's weight is much less at the age of 7–10 years than that of an adult, this excretion is relatively big. During the first 8 years of life, the basal excretion follows the weight curve (figure 3). The excretion is on the same level in eleven to fifteen year old children.

Of the total 17-hydroxycorticosteroids more are excreted per kilogram of body weight in children of all age groups than in adults (figure 4). Per kilogram of body weight this excretion during the first and second days of life is as high as in patients after surgical operation (HALME *et al.*, 1957). At the same time we must consider the greater metabolism, hydrolability and urine volume of children.

The excretion of total 17-hydroxycorticosteroids into the urine starts in our material earlier than the excretion of androgenic or estrogenic hormones (fig. 5) (NATHANSON *et al.* 1941, TALBOT *et al.* 1943). The increased excretion

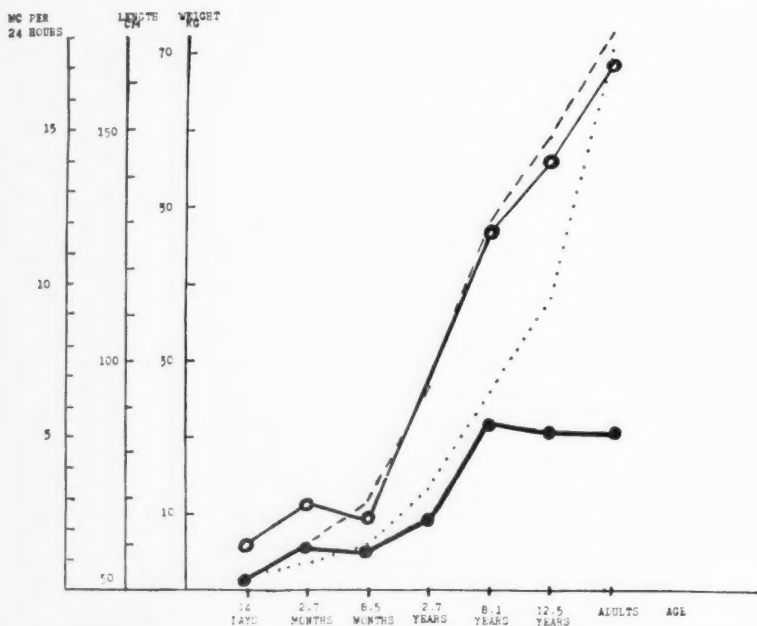


Fig. 3. The excretion of total 17-hydroxycorticosteroids into the urine (mg per 24 hours), in different age groups as compared with the length and weight curves of children.

The basal excretion = - - - - -.

The average excretion during the first and second ACTH-days = o—o—o—o—o

The length = — — — — —

The weight = ..... .

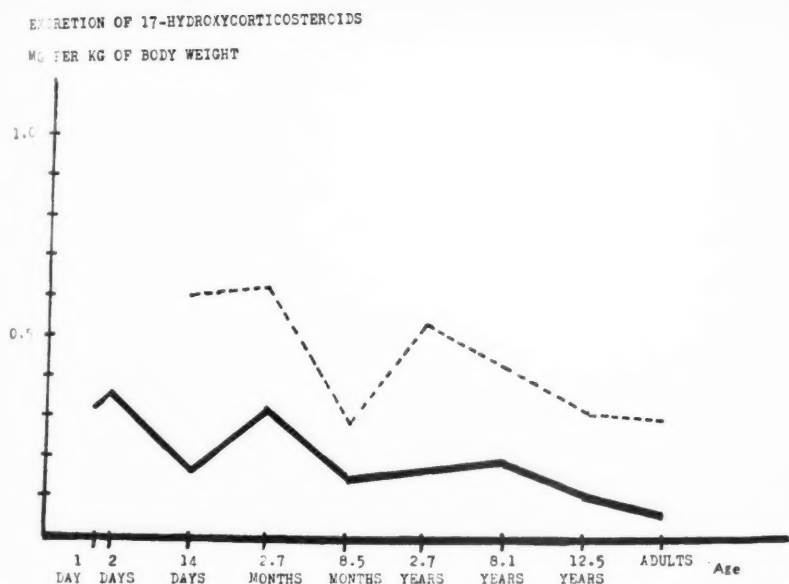


Fig. 4. The excretion of total 17-hydroxycorticosteroids into the urine (mg per kg of body weight in 24 hours) in different age groups of children. Comparison of this excretion with that of adults.

The basal excretion = —————

The excretion during depot ACTH-days = - - - - -

of androgenic and estrogenic hormones is connected with puberty and sexual maturity, and the development of the secondary sexual characteristics. The 17-hydroxycorticosteroids are excreted continuously beginning from birth. The excretion of 17-ketosteroids increases, however, clearly from the 9th year as that of estrogens in girls. Hydrocortisone is an important hormone for the maintenance of life during all its periods. A clear individual variation has been observed in the excretion of 17-hydroxycorticosteroids in children.

#### II. The Adrenocortical Reserve (figure 2)

The excretion of 17-OHCS during the first month is almost three times on the first ACTH-day (1.2 mg) and on the second ACTH-day (1.9 mg) more than four times the basal excretion. In this age group several of the children (6 cases) were prematures.

Children at the age of 1—6 months have an excretion during the first and second ACTH-days of 2.9 mg and 2.7 mg respectively, about twice the basal excretion in the corresponding age group.

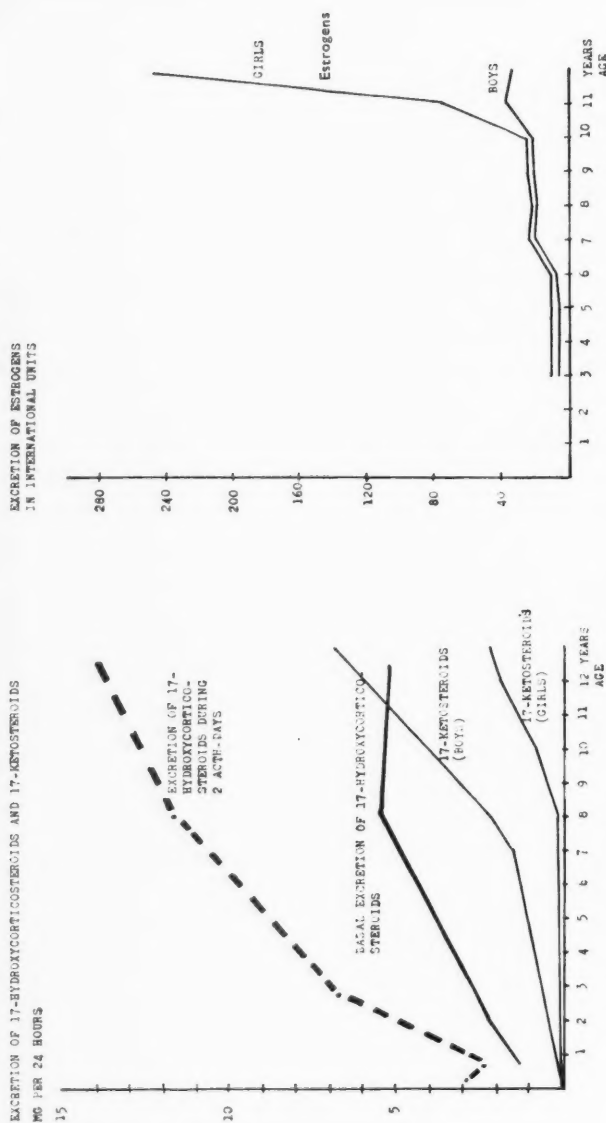


Fig. 5. The excretion of total 17-hydroxycorticosteroids into the urine (mg per 24 hours) as compared with the excretion of 17-ketosteroids and estrogens in different age groups of children (Nathanson *et al.* 1941, Talbot *et al.* 1943).

From the 6th to 12th month the excretion (2.4 mg) is nearly twice as great as the basal excretion during the first and second ACTH-days.

In the age group 1—4 years, the excretion on the first and second ACTH-days (3.5 mg and 4.8 mg respectively) is over two and three times the basal excretion.

In the group of 4—6 years the excretion on the first ACTH-day is 8.6 mg, and on the second 14.1 mg. It is clearly larger than in the previous age group. These values nearly correspond to the excretion in adult women with chronic rheumatoid arthritis during the first ACTH-day (PEKKARINEN *et al.* 1958). It is interesting to observe the good adrenocortical reserve at the age when children show an increased physical and psychical activity. In our material, there have been only 5 children belonging to this age group.

In the 7—10 years age group the excretion during the first ACTH-day is 10.7 mg, and on the second 12.7 mg or about twice as much as the corresponding basal excretion and, on an average, also larger than in the previous age group.

In the 11—15 year old children, the excretion during the first and second ACTH-days, 14.5 mg and 13.4 mg, is more than twice the basal excretion. Both in this and in the previous age group the average excretion of the two ACTH-days nearly corresponds to the excretion in adult women in the same test.

The adrenocortical reserve in children increases with age remarkably clearly even in the 4—6 year old children, just as the basal excretion of the total 17-hydroxycorticosteroids, and partly follows the length curve of the children. The size of the adrenocortical reserve is, however, in our material characterized by a considerable lability during the whole childhood, as well as many other biological phenomena in children. The excretion of total 17-hydroxycorticosteroids during the second ACTH-day does not increase in the groups of children as clearly as it does in the group of adults. This indicates the smaller total adrenocortical reserve in children. In adults we have found (PEKKARINEN *et al.* 1958) a continuous increase in the excretion of total 17-hydroxycorticosteroids also during the three consecutive ACTH-days.

#### SUMMARY

The basal excretion of total 17-hydroxycorticosteroids has been studied in urine altogether in 141 children from infants to 15 years of age and the adrenocortical reserve during a 2 days intramuscular ACTH-test of 0.55 units of depot-ACTH (Acton prolongatum. Nordiska Hormonlaboratoriet, Malmö) per kilogram of body weight twice daily in 63 children.

During the first and second days of life 1.0—1.1 mg of 17-OHCS are excreted. The basal excretion diminishes during the first month and increases during the

first year. At the age of 4—6 years it is 3.7 mg. At the age of 7—10 years the basal excretion can reach the level of adults, 5 mg.

The excretion of 17-OHCS of ACTH-days during the first month is almost three to four times and at the age of 1—4 years two to three times the basal excretion. The adrenocortical reserve in children increases with the age remarkably clearly as early as in the 4—6 year old children and approximately follows the length curve of children. In the group of 4—6 years the average excretion is during the first ACTH-day 8.6 mg and the second ACTH-day 14.1 mg, clearly bigger than in the previous group. At the age of 7—10 years it is during the first ACTH-day 10.7 mg and during the second ACTH-day 12.7 mg and in the 11—15 year old children 14.5 mg and 13.4 mg respectively. Per kilogram of body weight the basal excretion has been higher in children than in adults.

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### Studies of the Cerebrospinal Fluid Protein in Childhood

STEN WIDELL

Lund, Sweden

Published in *Acta paediat.* 47: Suppl. 115, 1958.

## Epilepsy in Children. A Clinical and Roentgenological Study

MARIT SKATVEDT, SIGURD EEK and ODD GARBORG  
Oslo, Norway

This study is based upon investigations concerned with 360 children suffering from epilepsy, made over a five-year period from 1950—1955. 200 were in-patients and 160 out-patients, and they ranged in age from 0—14 years. 188 were boys and 172 girls. Successful pneumo-encephalographic (PEG) investigation was carried out on 165 in-patients.

### *Summary and Conclusions:*

1. Of probable etiologic importance were following data: Seizures had occurred in the families of 28.7% of the 360 patients. 24% had a birthweight over 4000 gr. against 14% in the normal Norwegian population. Prematurity occurred in 9.5% and pathologic birth and perinatal complications in 15.5%.

2. 60% of the 165 pneumo-encephalographically examined patients suffering from epilepsy had pathological changes in their brains varying from slight to extremely severe.

3. The earlier in life the seizures started, the more frequent and severe were the pathological PEG findings. Thus 80% of the children where seizures started before 2 years of age (*in all 88 patients*) had abnormal PEG findings.

4. There is no clear correlation between the severity and extent of the epilepsy and the demonstrated brain injuries. Extensive pathological brain changes can be followed by a mild form of epilepsy and vice versa.

5. There is a similar lack of correlation between the PEG and the electro-encephalographic findings in these epileptic children.

6. In 40% of our patients where the PEG was pathologic, no etiologic factor causing brain injuries was found. It is reasonable to suspect that the damage in many cases had occurred during intrauterine life, as the symptoms were noticeable soon after birth.

The conclusion drawn from our investigation is that when epilepsy starts early in childhood a surprising number show organic brain injuries.

### DISCUSSION

*P. Plum, Denmark.* — (Questions Dr. Eek.)

- (1) What is the PEG like in hereditary conditions?
- (2) What is the PEG like in petit mal?
- (3) Is it possible to indicate a correlation between the PEG and the course of the disease?

*S. Eek, Norway.* — (Replying to Prof. Plum.)

Ad. 1. Patients with seizures in the family: Their PEG was the same as in the material as a whole.

Ad. 2. PEG in petit mal: Only one patient was examined, and showed a normal PEG. I have consequently no experience here.

Ad. 3. The therapeutical results in patients with serious brain changes demonstrated by the PEG were the same as in patients with minor cerebral changes or in those with a normal PEG.

If patients with marked PEG changes also had neurological symptoms the prognosis was poorer for the epilepsy too.

*M. Skatvedt, Norway.* — We have registered petit mal only in patients with short absences and 3/sec. bilateral synchronous spikewaves in the EEG. Some children have absences plus grand mal with 3/sec. spike-waves, others a cortical spike-focus + 3/sec. spike-waves. The group »pure petit mal» diminishes steadily.

### Cardiopathy in Friedreich's Ataxia

CLAES THORÉN

Stockholm, Sweden

Friedreich's ataxia is a heredofamilial, degenerative nervous disease with a monohybrid, recessive autosomal mode of inheritance. The fact that it may occasionally be connected with a heart disease has often been overlooked. From a patho-anatomic point of view a chronic and progressive myocarditis has been ascertained, involving diffuse, interstitial fibrosis, focal muscular necrosis, sparse inflammatory manifestations, and an undamaged endo- as well as pericardium. Fatty degeneration, sclerosis of the coronary vessels (NADAS 1951) and fibroelastosis (SOBERON 1958) have also been reported, observations that slightly confuse the pathologic picture.

Various murmurs have been noted and interpreted as functional. X-ray discloses a moderate cardiac enlargement. The cardiac symptoms may predominate to an extent that renders the nervous disease recognizable only secondarily. Subjective cardiac symptoms are noticed only in the late stages when decompensation and arrhythmias are the most conspicuous. Since the thirties, characteristic ECG-abnormalities have been recorded, representing the most common evidence of cardiac involvement.

EVANS, in 1949, suggested, in the course of his studies of familial cardiomegaly without neurologic disease, that isolated cardiopathy might occur in families

## FRIEDREICH'S ATAXIA AND CARDIOPATHY

## Pedigree of a family

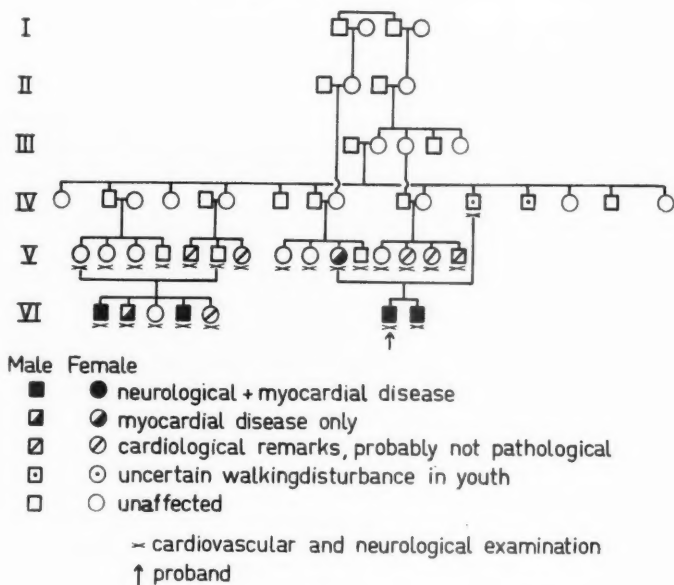


Fig. 1.

with Friedrich's ataxia. Further suspicion of this has emerged later, though stopping at mere assumptions (MANNING 1950, SOBERON et al 1955).

A family with not less than four intermarriages is demonstrated (Fig. 1). four boys are suffering from Friedrich's ataxia with cardiopathy. They show different stages of development, as can also be followed in the ECG. S-T and T wave changes can be provoked, or further emphasized, during exercise. In two of these cases the ECG led to the diagnosis of the basic disease. The ECG of the eldest boy, now 16 years of age, has been followed for 13 years. The changes show early progression and later a slow regression. Phonocardiographic studies are presented. In 3 of the cases almost identical murmurs are registered over the pulmonary region. This is a high-frequent, fairly strong end-systolic murmur, which has a certain stenotic appearance. The second sound is clearly split. The murmur is markedly accentuated during exercise and gives rise to thrill. This strengthening of the murmur remains even when the original heart frequency and duration of the systole has been restored. In the eldest boy a murmur was registered earlier that was interpreted as a definite pulmonary

stenosis. This had almost completely disappeared and instead, as a sign of cardiac dilatation, a pathologic gallop rhythm of the third sound was registered.

In this family there are two cases (aged 14 and 32) showing only signs of myocardial damage. Distinct ECG changes occurred, and in one of them also cardiac enlargement and a moderate systolic murmur. The cardiologic investigation included exercise ECG as well as determinations of the physical working capacity, the blood volume and the heart volume. Serum transaminases and lactic acid dehydrogenases were determined repeatedly, but gave normal values even in the 2 cases that revealed an ECG progression in process.

These two cases of isolated myocardial degeneration which have been demonstrated here may be said to confirm earlier assumptions. There is no doubt that the disease requires a homozygote genotype. The remarkable combination of degeneration in 2 quite different organ systems will necessarily engage several enzyme systems governed each by its own autosomal gene. In all likelihood, only one gene is defective in the purely nervous disease. The combination, which is reported in 30% (EVANS and WRIGHT 1942), would need another defective gene. Moreover, the latter defect might just as well occur by itself. This form of the disease has probably been disregarded earlier. Some of the cases of myocarditis which have previously been termed unspecific or idiopathic may have been genetic. It is conceivable, in the most lenient forms, that patients suffering from isolated cardiopathy may be heterozygotes and represent the actual carriers.

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## SESSION V

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## **Problems of Nutrition in Infancy**

### **Biochemical and Clinical Aspects**

O. MELLANDER and B. VAHLQUIST

Gothenburg and Uppsala, Sweden

Will in part appear in the commemorative volume to Professor Gino Frontali.

#### DISCUSSION

*K. Wilken-Jensen, Denmark.* — I wish to congratulate Prof. Vahlquist and his co-workers on the interesting investigations and the beautiful results obtained. From my point of view, it would be of interest to know if they have any information about the number of allergic diseases in the two groups. Prof. Glaser from Rochester, New York, claims that by giving soya bean milk instead of cow's milk to children of highly allergic families, he has obtained a significant difference in the occurrence of allergic disturbances in comparison with the occurrence in siblings fed with cow's milk.

Did you find any difference between the breast-fed and the artificially fed children as regards allergic disorders?

*B. Vahlquist, Sweden.* — We cannot count on our investigation material to throw light on the frequency of allergic conditions. Glaser's investigation has been severely criticized. It is retrospective and there does not seem to have been any representative control material. It appears to be a serious thing to advocate without any convincing evidence such a radical and potentially risky measure as the early introduction of soya «milk» as a milk substitute.

## **Prepuberty**

E. THAMDRUP

Copenhagen, Denmark

Puberty represents the dividing line between the period of pubescence and the period of adolescence. Puberty is often used synonymously with the menarche. Prepuberty is here used as the period starting with the growth acceleration of the gonads and ending with puberty.

### *The somatic development during prepuberty*

The somatic development in prepuberty is characterized by an accelerated growth of the body, and particularly of the reproductive organs.

On account of the great individual variations in the somatic development during this period, cross sectional studies often present an incomplete picture of the development, while longitudinal studies i.e. investigations where the same children are examined with short intervals during their growth, are more reliable (5).

It is shown that the growth acceleration during prepuberty becomes compromised in a material based on a cross sectional study.

The development of the reproductive system during the prepubertal period is characterized by growth of the genital organs and development of the secondary sexual characters (cf. fig. 1). There are considerable individual variations within the limits of the normal development. This applies not only to the age when the development starts, but also to the rate of the development and the order in which the different sexual characters appear.

Girls	Age in years	Boys
Growth of ovaries	8—9	
Vaginal changes:	9—10	
1. oestrogenization of epithelium		
2. Döderleins bacilli		
3. pH from 6—7 to 4.5		
Breast development		
Pubic hair	10—11	
Demonstrable genital growth	11—12	Growth of testicles
Pigmentation of areola	12—13	Growth of penis
Maximal height growth		Prostatic activity
Apocrine sweat glands		Pubic hair
Axillary hair	13—14	Transitory breast development
Menarche		Apocrine sweat glands
	14—15	Maximal height growth
		Voice changes
		Axillary hair-Moustache

Fig. 1. Pattern of normal (average) somatic development during prepuberty.

The most important features in the somatic development during prepuberty, the growth acceleration and the sexual development, are so closely combined (4) that they are presumably released by the same physiological mechanism, initiated by the development of the gonads as a response to the emission of gonadotropic hormones from the pituitary gland.

During prepuberty gonadotropic and gonadal hormones can be found in the urine in considerable amounts. The increased urinary excretion of gonadal hormones can be demonstrated about one year earlier than the excretion of gonadotropic hormones. This discrepancy may be explained by insufficient methods of analysis or perhaps by an increased function of the suprarenal cortex during the first phase of prepuberty.

Animal experiments tend to show that the emission of gonadotropic hormones from the pituitary gland is started from the hypothalamus (2).

*Factors influencing the somatic development during prepuberty.*

As the menarcheal age is so intimately associated with the other links of the sexual development, the results from menarcheal studies may be transferred to the development in prepuberty as a whole. The menarcheal age in a population is distributed according to the Gaussian curve (1).

*Genetic factors.* The importance of the genetic factors is most clearly demonstrated by the difference in age at the onset of puberty in the two sexes, but hereditary factors seem to influence the age of puberty, independent of sex. This transpires from studies of the menarcheal age in identical and non-identical twins (3, 6).

The rate of development of puberty and the sensitivity of the endorgans to the sex hormones are probably also dependent on genetic factors.

*External factors.* Studies of the menarcheal age variations in the different economic classes of the community point to the fact that the menarcheal age is influenced by external factors, primarily nutrition and weakening diseases.

During the last century, the menarcheal age seems to have fallen from the age of 16—17 years to 13—14 years in all the Scandinavian countries. Although the older methods of examination are encumbered with considerable uncertainty, it is probable that a marked decrease in the menarcheal age really has taken place, as the adolescent growth acceleration of the children, so closely related to the menarcheal age, also starts at an earlier age. Both phenomena are probably caused by external (nutritional-social-hygienic) factors.

Racial and climatic factors are apparently of little or no importance in comparison with the sociological and nutritional circumstances.

*Endocrine diseases.* The effect of these disorders depends on the affected endocrine organ and its influence upon the development of puberty.

The clinical manifestations may be characterized by precocious puberty, by sexual infantilism, and by an incomplete or abnormal development of puberty. Cerebral disorders influencing the hypothalamus and diseases in the pituitary gland, the gonads and the suprarenal cortex may represent the pathological lesion.

Abnormal sensitivity of the endorgans, primarily the mammarian tissue and the hair follicles, tend to provoke an isolated premature development of the mammary glands or the sexual hair.

Finally, the importance of continued longitudinal growth studies on children has been emphasized, preferably in centres where biological and biochemical metabolic studies may form a part of the research programme.

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#### DISCUSSION

K. Kaijser, Sweden. — An investigation of the occurrence of gonadal dysgenesis (Turner's syndrome) in Sweden, currently being undertaken in co-operation with Enell at Boden, with Söderhjelm at Skellefteå and with Kaijser at Eskilstuna, shows a considerable accumulation of cases in the northern parts of the country. It seems probable that this is dependent on genetic factors which manifest themselves more in the northern parts of Sweden where population movements are not yet as great as in the other parts of the country.

It would be interesting to obtain information on these conditions in the other Nordic countries.

#### Factors Affecting the Somatic Development of the Child: Social Environment

HOLGER HULTIN

Helsinki, Finland

The somatic development may be divided into two parallel concepts, that is, growth in itself, which means increase in mass, and development, which is defined as increase in complexity. These two growth processes will be discussed together, because of the continuous two-way effect they have upon each other.

Growth and development depend on genetic and environmental factors. The *social environment* of the child is part of the latter group.

The social environment consists of a social and an economic component, which two are completely interdependent in this connection. The social component of the environment is regarded as related to the association of man with his fellow man, and since economic life is a chief determinant of social existence in our society, it seems appropriate to consider these together. The pure social environment may, however, not always follow the economic status, especially when the level of education is the determinant of social acceptance.

In the following, the author describes a number of conditions of the child, in order to establish their ill effect upon normal growth and development. Thus it will be possible to find, indirectly, criteria of the influence of the social environment upon the somatic development of the child.

#### *A. Disease*

SYDENSTRICKER gives a broad classification of some of the social and economic factors that might be involved in health and disease. Almost any kind of disease tends to interfere with continuity of growth, depending perhaps more on duration of illness than on severity. (LEAVELL, CLARK & al.)

According to published works, there is a clear association of illness with low income, of disability with poor economic status, of specific disease mortality and infant mortality with poverty.

The problem of children of working mothers is discussed in more detail, and the latest figures from Finland are given. (See: Social facts about children in Finland, Publication n:o 28 of the Central Union for Child Welfare in Finland).

The incidence of disease is higher among children in day care than among those taken care of in their own homes. This is with the exception of children coming from the poorest social surroundings. (FRISELL; NORDENFELT; BJUGREN, KRAEPELIEN & LIND; WEGELIUS). Children under 2, when taken care of by different persons, often show a retardation of the somatic development, without signs of any known disease.

The demand for hospital beds was investigated in Helsinki 1953-54 by V. RANTASALO & H. VALPOLA. The material, consisting of infants and children under 15 years, was divided into 4 categories according to the socio-economic status of the parents. It was shown that the demand for hospitalization due to infectious diseases was greatest in the two lower social groups, and this was particularly true of the 0-2 yrs age group.

### *B. Retardation of Increase in Weight and Height as a Result of Poorer Social Environment*

Reference is here made to the thesis of KATRI MALMIVAARA, dealing with the weight and height increase of infants and children under 14 in Helsinki during the second World War. In this publication, there is given a comprehensive summary of investigations carried out to find the correlation between socio-economic status and growth in height and weight of the child. As a brief conclusion of these studies, it may be said that although the genetic factor is in the first hand responsible for the growth of an individual, the social environment, however, has a very important influence upon the rate of growth and thus upon the end result. If the socio-economic status is bad, the negative effect on the growth and development is inevitable, and here the deficient nutrition is perhaps the main factor.

### *C. Prematurity*

Prematurity is a growth-disturbing factor of the first degree. According to an investigation carried out in Great Britain in 1946, there is a significant correlation between birth weight and social status. The rate of premature births is the higher, the lower the socio-economic status. The same is true of housing facilities. With a decreasing social environment comes an increasing frequency of pregnancies. The risk of a premature birth is at a minimum with an interval of 2 to 6 yrs between the pregnancies. (J. W. B. DOUGLAS).

In the U.S.A., the Negro infant tends to have a lower birth weight than the White newborn. The corresponding figures are about 2,350 g (Negro) to 2,500 g (White) (SCOTT, JENKINS & CRAWFORD). It has been thought that this is due to a genetic difference of races, even though this has not been proved. With a rising socio-economic standard of the Negro population in the U.S.A. this difference in birth weight has started to disappear, and in any case thus appears to be of environmental origin. (C. A. SMITH).

### *D. Infant and Child Mortality*

The ultimate, fatal consequence of a negative influence upon the growth and development is the death of the child.

Mention is here made of the Public Health measures which are possible in order to reduce the mortality rates, but it is also pointed out how the rising standard of living, with a higher socio-economic status of the population, brings with it the hazard of death in traffic. The leading cause of death among our children is constituted by accidents of various kinds.

### *E. Caries*

The incidence of caries in the child population, and the social environment, show an inverse association. This statement is made with a number of important reservations. The lower incidence of caries in children of poor social circumstances, does not, however, put these children in a better position compared with their more well-to-do fellow children because of the neglected dental care in the first instance. The result is that in spite of a lower incidence, caries interferes with normal growth mostly in the lower socio-economic population group.

As a summary of the above criteria in relationship to the social environment of the child, it can be said, that the quality of this environment usually shows a direct association with the somatic growth and development of the child. The lower the socio-economic situation, the higher the number of adverse effects upon the growth.

Furthermore, it is true that high economic status, and a rising standard of living, do not *as such* assure good health, since some people with ample income may purchase goods and services that impair health.

### DISCUSSION

*H. Lichtenstein*, Sweden. — The social milieu is of great significance for the functional development of the child.

The Swedish social classification is not adequate for compiling the results of our longitudinal study and we have employed the 5-point scale worked out by Professor Graffar, Brussels. We have also endeavoured to construct a social profile using Graffar's scale.

## **Iron Requirements in Infancy**

M. SEIP

Oslo, Norway

Studies on the amount of stainable iron (hemosiderin) in bone marrow punctates from healthy infants indicated that the congenital iron reserves are usually adequate to meet the demands for about 4 months after birth in full term babies. From this age, the stainable iron disappears from the bone marrow, as a sign of threatening or fully developed iron deficiency.

A large series of «healthy» infants on a diet with a calculated iron content of 3 mg./day at 6 months, increasing to 6 mg./day at 12 months of age, showed

a marked drop in mean hemoglobin levels from 5 months of age. Fullblown iron deficiency anemias were not at all rare, and mild hematologic signs of iron deficiency were extremely common. With a greater iron intake, there can be prevented the secondary decrease in hemoglobin levels from about 5 months of age.

The recommended daily allowance of 6 mg./day (National Research Council) will for many infants be insufficient to prevent iron deficiency. As, on the average, less than 10 per cent of food iron is absorbed by healthy infants, the daily intake should be as high as 10—15 mg. This is best obtained by the administration of adequate quantities of vegetables, meat, liver, and enriched cereals from the fourth month of life.

#### DISCUSSION

*B. Vahlquist, Sweden.* — The problem introduced by Seip is a timely one. Lichtenstein and his associates made important contributions to it. It can now be said that all the evidence goes to show that from six months of age the child suffers for a long time from iron deficiency, often latent but sometimes obvious in lowered hemoglobin values. The latter observation was a frequent one in Sweden when I made my investigations at the beginning of the 1940s. It may be that the frequency of a manifest iron deficiency varies in the different Nordic countries. Further examination of the suggestion put forward by Seip might be justified in any case.

*C. Friderichsen, Denmark.* — The investigations of Professor Vahlquist and Dr. Seip confirm a clinical observation which I made some forty years ago when I experimented with Lichtenstein's therapy with large doses. If large doses of iron were given to a child suffering from anorexia, discomfort and poor weight gain but with a normal hemoglobin, the weight increased considerably though exactly the same diet was maintained.

I believed at the time that the iron functioned as a catalyst in digestion. We have been shown today what happens in the organism. I have always employed iron prophylactically and learnt to give it in parallel with ADC vitamin. It is my definite impression from a nursery that infections are fewer among the children given iron prophylactically.

## **General Meeting of the Northern Pediatric Association on July 2nd, 1958.**

On behalf of the former Secretary General, Dr. ARNE NJÅ, who was unable to attend the meeting. Dr. H. HAGELSTEEN, Norway, gave an account of the financial status of the Association.

The General Meeting voted unanimously to adopt changes in the Statutes of the Association as proposed by Dr. JUSTUS STRÖM, Sweden, at the meeting in Oslo in 1954. The statutes in their present form are published as an appendix to these proceedings.

As the passing away of Professor Y. ÅKERREN, Sweden, had left his seat on the Board vacant, and after the retirement of Professor P. HEINIÖ, Finland, and Dr. A. NJÅ, Norway, the following new Board was elected:

### *Denmark:*

P. PLUM  
A. ROTHE-MEYER  
O. ANDERSEN  
E. W. FLENSBORG (Secr.)

### *Finland:*

C.-E. RÄIHÄ  
T. SALMI  
N. HALLMAN  
B. LANDTMAN (Secr.)

### *Norway:*

L. SALOMONSEN  
A. SUNDAL  
R. RINVIK  
H. HAGELSTEEN (Secr.)

### *Sweden:*

S. SIWE  
J. STRÖM  
B. VAHLQUIST  
J. LIND (Secr.)

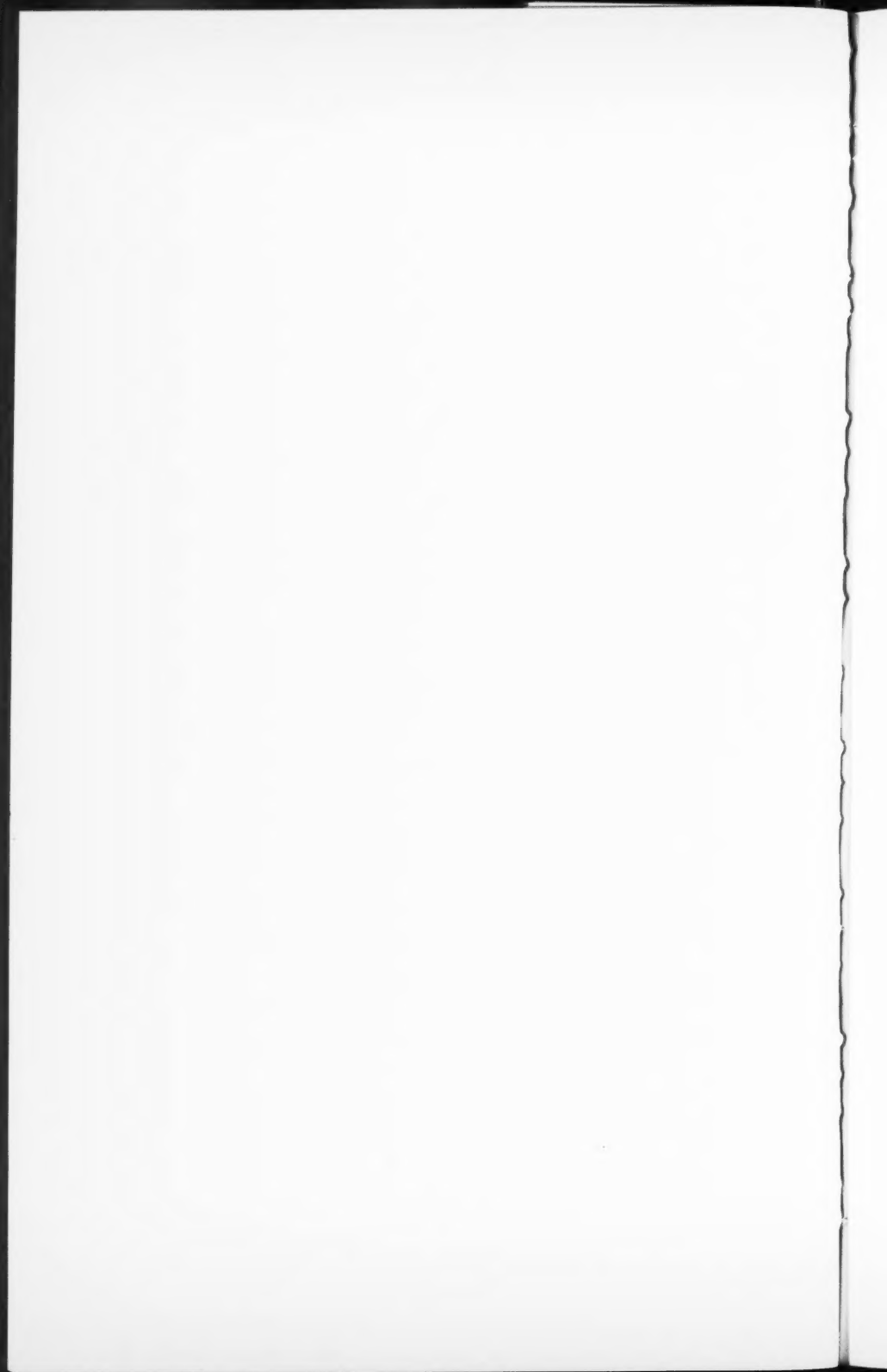
As Auditors of the Association were elected:

Denmark: E. GJORUP  
Finland: S. LAHDENSUU  
Norway: HANNA BERGHOFF  
Sweden: B. BROMAN

On the motion made by the Board, the general meeting decided unanimously to make Professor NILS MALMBERG, Sweden, an honorary member of the Association.

On the motion made by the Board, the General Meeting decided that the next congress be held in Copenhagen in 1961. Professor P. PLUM was elected president of the next congress.

The President, Professor C.-E. RÄIHÄ, closed the meeting.



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## **1. Determination by Means of a Bicycle-Ergometer of the Physical Working Capacity in Children**

F. ADAMS, E. BENGSSON, H. BERVEN, M. BÖRJESON, I. ENGSTRÖM, D. IKKOS,  
B. JONSSON, P. KARLBERG, and S. KRAEPELIEN  
Stockholm, Sweden

The exercise tolerance test, ad modum Karolinska Sjukhuset, has been applied to determine the physical working capacity in both healthy and sick children. The studies were carried out at different laboratories in Stockholm. The technique of the exercise test and the physiological background are described (see references).

The material consisted of the following groups:

I. Healthy children in the age group 5—14 years, 227 boys and 190 girls.

The working capacity is correlated to age and body size (weight and height). A difference between sexes is apparent, with the boys having a higher working capacity than the girls. The variations among the normal children are shown in a 95% confidence interval in relationship to body weight. The heart volume was also determined in 200 of these children, for it has been proved that the relationship between working capacity and heart volume is useful in practical clinical judgement.

II. Working capacity in healthy children both before and after the summer vacation was determined for a group of normal children. Those children who had before the summer, within the limit of variations for normal material, a low working capacity, showed a higher increase in working capacity after the summer than those who had already had a good working capacity before the summer.

III. Overweight children.

The material consisted of 24 nine year old boys. They showed a low working capacity in relationship to body weight, heart volume, and total amount of hemoglobin, as compared with children of the same age with normal body constitution.

IV. Children with congenital heart diseases.

The working capacity was determined in cases with a left-right shunt, in cases with a right-left shunt and in pulmonary stenosis cases without shunt. In young individuals, the working capacity may be high in cases of a large left-right shunt, but the working capacity will decrease with an increase in age (over 25 years).

The working capacity is always low in cases with a right-left shunt. Even patients with a high degree of pulmonary stenosis may have a normal working capacity. The causes of the variations in working capacity in different individuals are shown in studies of the effect of work on the hemodynamics of the heart in cases with congenital heart diseases.

#### V. Children with acute myocarditis.

Patients who had been hospitalized for myocarditis were examined about one month after discharge from the hospital. At that time they showed a low working capacity in relationship to body weight and heart volume.

The decreased working capacity in relation to body weight is an expression of decreased stroke volume in cases where the heart's ability to contract is impaired.

If the impaired ability of muscular contraction also produces an increase in heart size, the largest differences will be found in the relationship of working capacity to heart volume. The relationship was shown to be most useful in cases of acute myocarditis.

#### VI. Symptom-free asthmatic children.

Twenty nine children were examined in a symptom free interval. The physiological working capacity showed a tendency to be decreased to some extent in relation to the body weight, but this did not prove to be statistically significant.

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## 2. Relationship Between Physical Ability and Growth in Children

J. GHESQUIERE

Louvain, Belgium

## 3. The Determination of the Water Content of Biological Samples by the Karl Fischer Method

PAAVO MÄKELÄ and JOUNI PURANEN

Helsinki, Finland

The authors present some results of water content determinations of biological material. The water content of the blood plasma and red cells was determined as has previously been described. (Puranen et al.). The determination of the water content of rat muscle tissue was made by titrating with the Karl Fischer reagent an aliquot of a methanol extract of the ground tissue specimen. The results have been compared with the results of an oven drying method.

The water content of the plasma was determined in 183 cases. In 151 cases, i.e. in 82.6 per cent, the samples had a water content between 92—94 per cent (v/v).

In erythrocytes, the water content was determined in 180 cases. 125 cases (= 69.4 per cent) had a water content between 72—74 per cent (v/v).

The standard deviation of the method employed was  $\pm 0.4$  per cent.

The determination of the water content of the muscle tissue of rats was

made in 38 cases. The mean of these determinations was, by using the titrimetric method, 77.8 per cent (v/v)  $H_2O$ , and, determined by the oven drying method ( $10^\circ C$ , 24 hrs.) 76.5 per cent, respectively.

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### 4. A Method for the Continuous Recording of Movement

PAAVO MÄKELÄ, ERIC IVAR WALLGREN and KALLE ÖSTERLUND  
Helsinki, Finland

A report is given of the application of a method capable of recording movements on the basis of the changes of electrical capacitance. The device is suitable for recording the pattern and frequency of movements of different types. The amplitude of the movement can be recorded only if the device is calibrated separately for each recording. The most important advantages are the elimination of slowness, elasticity, friction and mass, and thus the recording can be regarded as a fairly accurate projection of the movement. The device is suitable for the recording of various movements, e.g. pulse, breathing and the apex beat.

### 5. Autoradiographic studies on the Distribution of $I^{131}$ -Labelled Proteins in Living Human Fetuses

YRJÖ PARTANEN  
Helsinki, Finland

Eighteen fetuses, presumably normal, and one anencephalic monster, were obtained in Stockholm on legal abortion, and injected with protein hormones iodinated with  $I^{131}$  (about 200  $\mu c$  per fetus). Control experiments were carried out with human and bovine serumalbumin and inorganic  $I^{131}$ .

On operation, the cord was prolapsed before delivery of the infant. The injection was made into the umbilical vein. Three to twenty minutes after injection, the fetuses were frozen in an acetone-dry ice mixture with an initial temperature of about  $0^\circ C$ , and dropping to  $-70^\circ C$  during about 20–30 minutes. The entire frozen fetus was then set in moist cotton, frozen to a block, and sectioned on a microtome in a cold room ( $-12^\circ C$ ).

The slices were taken on »Scotch Tape» and after freezedrying put on x-ray film. After a suitable time of exposure, the films were developed and the slices stained with hematoxylin-eosin.

The fetal zone of the adrenal gland proved to be of special interest, because it behaved differently in relation to some gonadotrophic hormones (CG and LH) than the other tissues.

## 6. The Analysis of Amino Acids by High-Voltage Paper Electrophoresis

J. K. VISAKORPI, J. PURANEN and ANNA-LIISA PURANEN  
Helsinki, Finland

The authors give here some results of the applicability of high-voltage paper electrophoresis in the analysis of amino acids, especially for clinical purposes.

For the present, the method is little used, this obviously being due to the high price of the necessary apparatus. In the Children's Clinic of Helsinki, attempts have been made during the last two years to apply this method in clinical analysis. (Visakorpi, J. K., Puranen, Anna-Liisa; Puranen, J., Puranen, Anna-Liisa, Hallman, N.).

This method and the usual paper electrophoresis have the same principles, but the voltage employed is higher in this method, being about 50 V/cm. The heat produced is removed by  $-13^{\circ}/15^{\circ}\text{C}$  liquid cooling. A great advantage is that in comparison with usual paper chromatography analysis, one does not need to remove electrolytes from the biological fluids before the electrophoresis. The proteins have to be removed, and this has been done by ultrafiltration. The analysis is made on a paper strip 1 m. long and 15 cm. wide. The running time needed to separate amino acids is about 1—3 hours; the fastest amino acids move about 90 cm. in 3 hours with the use of a potential gradient of 50 V/cm. The buffer employed was an acid one, with a pH of about 2. With this pH, all the amino acids get a positive charge. By this method the separation of different amino acids in biological fluids is satisfactory. Semiquantitatively, the amino acids can be estimated photometrically by elution in methanol of the amino acid spots stained with ninhydrine, and cut from the pherogram.

Some results of the analysis of normal biological fluids are given here.

*Urine.* In a quantity of normal urine, containing 0.250 mg total-N (Kjeldahl) you can clearly find the following amino acids: taurine, glutamine, serine, alanine, glycine, histidine, and also, weakly, tyrosine, phenylalanine, asparagine, threonine,  $\beta$ -aminoisobutyric acid and lysine.

*Serum.* The following amino acids separate from 100  $\mu$ l of serum: glutamine, leucine and isoleucine together, valine, serine, alanine, lysine, and more weakly, histidine, glycine, threonine, proline, phenylalanine and tyrosine.

*Cerebrospinal fluid.* The pherogram made from 100  $\mu$ l of CSF is in general the same as that made from 100  $\mu$ l of serum, but it contains more glutamine.

*Sweat.* Sweat is rich in amino acids. In 30  $\mu$ l of sweat, you can very clearly find serine, alanine, and lysine, clearly glutamine, threonine,  $\beta$ -aminoisobutyric acid, citrulline, glycine; and weakly, tyrosine, cystine, leucine, histidine and lysine.

In addition, the authors give the pherograms of three pathological urines.

Case 1 is that of a three year old boy with a diagnosis of pneumonia. In his pherogram, you can clearly see a cystin-lysinuria, while the excretion of arginine and ornithine is quite normal. The excretion of lysine was 200 mg/24 hours and of cystine 100 mg/24 hours. The patient had no symptoms of this disease.

Case 2 is that of an infant of 4 months of age who had during his whole life been icteric. The diagnosis was congenital hepatitis. The total excretion of amino acids was increased. In the pherogram one can see seven almost equal spots caused by the distinctly increased excretion of serine, threonine, lysine, and the slightly increased excretion of glutamine, glycine, alanine, and histidine. In this case, the amino-aciduria had arisen probably from the D-hypovitaminosis, which pherogram is very much like this one. The pherogram of this case is not typical of the pherogram of a liver cirrhosis.

Case 3 is that of a 12 year old girl who had from the sixth year of age suffered from a collagen disease (lupus erythematosus disseminatus) and who had been treated over a long period with cortisone. She had a very heavy general amino-aciduria, and the excretion of amino acids was about 30 times greater than normal. In the urine there were in particular secreted the essential amino acids found in high concentrations in the serum, i.e. leucine, isoleucine, valine, serine and lysine. The girl had evidently an almost complete deficiency of tubulus reabsorption.

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## 7. A Longitudinal Study of Children's Biological Development in a Modern City Community

P. KARLBERG, G. KLACKENBERG, I. KLACKENBERG-LARSSON,  
H. LICHTENSTEIN, I. SVENNBERG and A. WALLGREN  
Stockholm, Sweden

A longitudinal study of children's development, physical as well as psychic, has been in progress since 1955 at the Pediatric Clinic, Karolinska Sjukhuset. This is a part of the Co-ordinated Growth Studies from The International Children's Centre in Paris. Such a study has been carried out in London since 1951, Paris — 1953, Zürich — 1954, Dakar — 1954, Kampala — 1954, Brussels — 1955 and Louisville — 1957. Contact between the various study centres has been maintained primarily through yearly conferences supported by the International Children's Centre.

The Swedish material has been recruited mainly from the ante-natal clinic in Solna, a suburb of Greater Stockholm, fairly close to the Hospital. During the period April 1955 to March 1958, 213 children were included in the study.

The children are examined at the ages of 1, 3, 6, 9, 12, 18 and 24 months, and later once yearly. The physical as well as the psychic development is followed and also the social background (for details see earlier publications). The various factors investigated, and the methods employed, were presented in the exhibit, and in addition some preliminary results were given.

The purpose of the study is to obtain as true a picture as possible of the complicated interaction of soma and psyche and constitution and environment. By continuous annual examinations of the gradual maturation of body and psyche, and their developmental tendencies in children whose environment we know from both a social and a cultural aspect, a picture is formed through the eyes of various specialists of the probable essentials in the chain of causality.

Earlier definite observations of the individual cases can be correlated with what appears later. The relation between body build and the psychic structure will be investigated. The pattern of reaction which has prognostic value can be separated from that pattern which is coincidental. Perhaps we can learn something about the possibilities of predicting at an early age the risks a child will run during growth. The concept of health and normality will be clearer.

It should be possible for the investigation to give valuable viewpoints in the actual discussion of the frequency of testable aptitude levels in different social classes, and of the variability of aptitude as a consequence of cultural influences. The international comparison between widely different cultures and environments will further provide relief to the results found.

Preventive child care and the clinical evaluation of children should receive an essential broadening of their basic knowledge, and a deepened insight into the mechanisms important to human growth and development.

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### 8. Investigation on the Development of Finnish Children

NIILLO HALLMAN, ARVO LEHTOVAARA, KALEVI KOSKI, RITVA TUISALA  
LEENA JÄRVINEN, and JOHANNES HAATAJA  
Helsinki, Finland

The investigation group known as »Healthy Children» has since the year 1953 carried out an investigation which clarifies the development of Finnish children. The investigation is divided in the following main lines:

1. Observations of the physical development.
2. Observations of the psychic development.
3. Investigation on the teeth and the anthropological measurements.

The material is divided into:

- The basic material (100 children) which involves close observations of Helsinki children.
- The additional material, consisting of 3000 children, who are chosen by the employment of appropriate statistical methods. This material represents the whole country. Phases of the investigation were separated by an interval of three years in order to find out the longitudinal development.

In the exhibition, a few scattered items of information on the more interesting data are represented, for example the height, the weight, the amount of hemoglobin and the frequency of dental caries in children, and some results of the psychological tests.

### **9. Concerning the Postural Faults of Elementary School Children in the Town of Turku, Finland**

MATTI DAHL  
Turku, Finland

A posture examination has been made of 4,524 elementary school children in the town of Turku in Finland.

Of the pupils 2,208 were girls, and 2,316 boys.

The material was divided equally among the schools in the centre of the town and in the suburbs.

On an average, 35 per cent had good posture and about 65 per cent faulty posture.

No clear difference between the sexes could be noticed.

In upper classes there were 5 per cent more postural faults than in lower classes.

In upper classes the faults were worse than those in lower ones.

Local stiffness in the lower part of the thoracic spine, evidently caused by rickets, appeared in 18 per cent of the examined children.

Severe postural deformities could be found in about only 2 per cent of this material.

In addition to the spine, deformities of the thorax and the extremities were also examined.

About 20 per cent of the children had Harrison's groove, and about 25 per cent weak foot.

About 10 per cent were knock-kneed.

The length of the way to school had no influence on posture.

The pupils with good posture had a mean value of marks in gymnastics of 7.58, and those with poor posture 7.42.

### **10. Respiratory Studies in Newborns**

P. KARLBERG, G. KOCH, G. WALLGREN and F. GEUBELLE  
Stockholm, Sweden

The various components of the lung function were illustrated, including lung volumes, the mechanics of breathing, minute volume and alveolar ventilation, functional dead space, gas exchange with diffusion relationships. The apparatus and methods of study of these functions which are applicable to newborns were described.

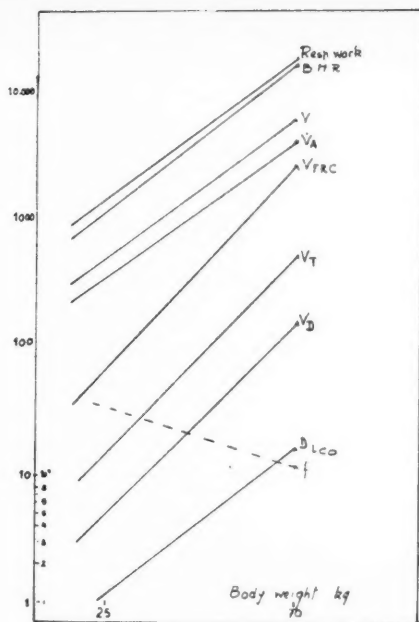


Fig. 1.

A summary of the values found for the different components for healthy newborns of varying ages, as well as for newborns in «respiratory distress», was given, representing the results of a series of different investigations (of which a part have already been published elsewhere).

The exhibit was summarized in the following manner:

In the transition from intra- to extra-uterine life, the child must within a very short period of time establish a lung function adequate for oxygenation of the blood and the disposal of carbon dioxide. Respiratory studies, especially with respect to the relationships of the mechanics of breathing, show that the lung function is developed most rapidly during the first few breaths and the following minutes of life. From the age of some hours up to the age of one week, lung function develops less rapidly. For this reason, this age group can be taken as a unit for comparison with adults (see Fig. 1).

Lung volumes: functional residual capacity, tidal volume and functional dead space increase proportionally per kilogram body weight. On the other hand, however, functional values such as basal metabolism, minute volume and alveolar ventilation, respiratory work and diffusion capacity are proportionally greater per kg body weight in newborns than they are in adults.

The demand on the function of the lungs during the newborn period is thus

greater in relation to the dimensions of the lungs than it is later in life. The lungs must be utilized more intensively and the respiratory frequency must therefore be higher.

The newborn's higher respiratory frequency follows Otis-Fenn-Rahn's law in that the frequency and tidal volume are adjusted in such a way that the necessary alveolar ventilation is maintained with a minimum of work (demonstrated by Cook *et al.* 1957). The work per minute volume is also approximately as great as it is in adults.

In »respiratory distress» the demand on functioning lung parenchyma in relation to lung size is even greater, with a higher respiratory frequency as a consequence. Despite the fact that the principle of economy is also valid under these conditions, up to 10 times as much work per minute volume is demanded as in healthy newborns and adults. In »respiratory distress», the respiratory frequency is a good indication of the extent and severity of the lung changes and clinically should be registered regularly.

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## II. Estimation of Metabolism in the Newborn

JOHAN TALLQVIST and C.-E. RÄIHÄ

Helsinki, Finland

In order to solve this task, we have concentrated the greater part of the work upon the problem of the heat energy determination. This should not be interpreted by assuming that the other estimations are of secondary importance, but since investigators elsewhere have already for some time been working on the estimation of respiratory gases in this connection, we have used results and constructions already attained by others.

For the estimation of the heat production in the newborn, we have constructed what is termed a differential calorimeter, which is composed of two thermally isolated cylinders (see fig. 1) in which space A is called »space of measure-

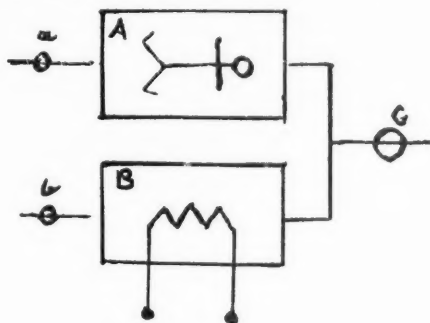


Fig. 1.

ment» and space B »space of reference». Two identical atmospheres are conducted through these cylinders, and both temperature and humidity are measured at the point of infusion (point C) and at the points of exhaustion (a and b). In addition, a source of heat with a regulated heat production is placed inside the »space of reference». This source of heat is composed of an electric element, fed by an amplifier in such a way that the temperatures at points »a» and »b» are equal. Thus, if the conditions in spaces A and B are similar, the heat produced by the element in space B equals the heat produced by the infant in space A. The efficiency in B can readily be determined by measurement of the current in the battery. The efficiency E expressed as Kcal/hour is

$$E = 0.86 \left( \frac{i}{[A]} \right)^2 \cdot R$$

$$\left[ \frac{\text{k cal}}{\text{h}} \right] \quad [\Omega]$$

The composition of the atmosphere in the calorimeter is regulated by taking suitable amounts of  $\text{CO}_2$ -free air, oxygen and  $\text{CO}_2$  from available gas containers. The gas mixture is conducted through a humidifier, which is shown in the figure.

The dry, warmed, incoming gas mixture (see fig. 2) is conducted through the regulating valve (V) into the water-container (F) and further into the calorim-

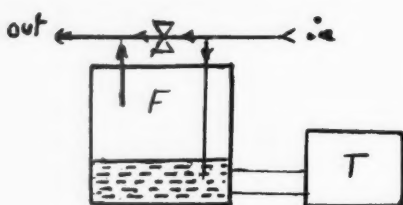


Fig. 2.

eter where the relative humidity of the gas mixture can be up to 100%. The ultrathermostat (T) keeps constant the temperature of the water in the humidifier.

The gas mixture is divided into two equal parts, of which one is conducted through the space of measurement and the other through the space of reference. When the gas has passed the calorimeter, its temperature, humidity, and content of  $\text{CO}_2$  and  $\text{O}_2$  are determined. The  $\text{CO}_2$  is analysed by means of a URAS I  $\text{CO}_2$ -analyser which has been modified in such a way that the  $\text{CO}_2$ -content of space (A) is compared with the  $\text{CO}_2$  content in space (B), and hence the  $\text{CO}_2$ -analyser directly measures the  $\text{CO}_2$  production of the infant.

All the results, temperature, humidity, efficiency etc. are registered by a Philips 12-point registrator, and can thus all be read on the same paper.

## 12. The Iodine Content of Human Milk in Finland

PIRKKO LAHESMAA and PANU VILKKI

Turku, Finland

The iodine content was determined in samples of mother's milk obtained from two areas of Finland which differ in the incidence of goitre. 107 milk samples from mothers in the non-goitrous Turku area, and 18 samples from mothers in the goitrous Kuopio area, were followed for their iodine content by day and night, in different seasons of year, and in different phases of lactation.

The average iodine content of mother's milk was found to be:  $53.3 \pm 1.4$  micrograms per litre in the non-goitrous area (Turku)  $25.1 \pm 1.6$  micrograms per litre in the goitrous area (Kuopio). The difference between the two averages is highly significant.

No correlation was found between the iodine content of milk and the level of protein-bound iodine in the serum of the mother.

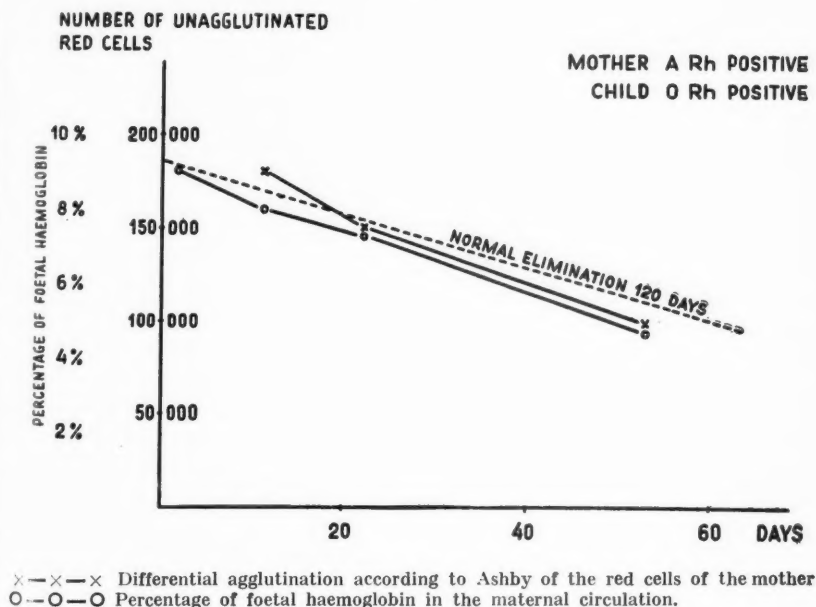
The analytical procedure was an alkaline combustion method described in a previous paper of one of the authors (Vilkkil, 1956).

### 13. A Case of Foetal Bleeding into Maternal Circulation

H. R. NEVANLINNA

Helsinki, Finland

In neonatal anaemia, the suspicion of foetal bleeding into maternal circulation as an aetiological factor can only seldom be proved. The case to be presented gave a possibility, with the aid of two different laboratory methods, of making a correct diagnosis, and in addition of making a calculation of the amount of foetal blood lost through this mechanism.



*Case history:*

The mother was 25 years old, primigravida in good health, and with a normal course of pregnancy. No bleeding *ex utero* was observed during pregnancy and the onset of labour. She belonged to group A Rh positive, no abnormal antibodies were found during and after delivery.

After a spontaneous delivery, the child, a boy of 3,400 grams, was observed to be pale but otherwise vigorous. At the age of 8 hours, the capillary blood showed a haemoglobin content of 6.7 g per cent with 2.1 mill. red and 25,000 white cells. There were 33 nucleated red cells per 100 white cells, and the reticulocyte count was 16.5 per cent.

The child received three transfusions with 40 cc of packed red cells each and made an uneventful recovery. He was not found to be jaundiced, the total bilirubin content at the age of one day being 2.4 mg per cent.

The child belonged to group O Rh positive, Coombs direct test was negative. Two days after delivery an Ashby count and foetal haemoglobin determination were made from the mother's blood, and the values were followed as shown in the diagram several times subsequently.

When one takes into account the difference between the blood volume of the child and mother, it seems that the foetus had bled into the maternal circulation a quantity of blood which was about equal to his own blood volume.

#### 14. To What Extent Can Prematurity Be Prevented?

C-E. RÄIHÄ, C. E. JOHANSON-UNNERUS, J. LIND, U. WEGELIUS, A. BACKMAN,  
J. KIHLEBERG and P. VARA  
Helsinki and Turku, Finland  
Stockholm, Sweden

The correlation between the maternal heart volume, the birthweight of the baby and the frequency of prematurity has been studied in about 2,000 cases. The material consists of three groups selected at random, twins being excluded:

A. The maternal heart volume measured the day after parturition in 203 cases resulting in premature births (child weighing  $\leq 2,500$  g), and in 578 cases resulting in full-term births (child weighing  $> 2,500$  g). In this material, ten per cent of the fullterm and 15% of the premature babies were delivered by mothers with either albuminuria or hypertension during pregnancy.

B. The maternal heart volume measured during pregnancy only, in 520 cases, of which 15 resulted in premature births. (U. Wegelius.)

C. The maternal heart volume measured during pregnancy and after parturition in 596 cases. In 72 of these cases, where the maternal heart volume was  $\leq 320$  cc/m<sup>2</sup> of body surface, the mothers were ordered not to do heavy work and to rest some hours every day.

### Results

A. In two thirds of the pregnancies resulting in premature birth the heart volume of the mother was below 600 cc. In the pregnancies resulting in full-term births, on the other hand, only one third of the mothers had a heart volume below 600 cc. The percentual risk of premature birth decreases by 50% for every increase of 100 cc in the heart volume.

The correlation between the size of the placenta and the maternal heart volume is not as evident. (Backman).

B. The size of the maternal heart and the increase in volume during pregnancy is in close correlation with the birth weight of the expected baby (figure 3).

C. In the group of 72 pregnant mothers who had been ordered not to do heavy work and to rest, only one baby with a birth weight of 2,500 g was born, this after a gestation time of 292 days. In these 72 cases, the mean maternal heart volume during pregnancy equals that found in material B for the mothers giving birth to premature babies. Out of 22 babies born before the 250th day of pregnancy in material C, only 9 have a birth weight  $\leq 2,500$  g.

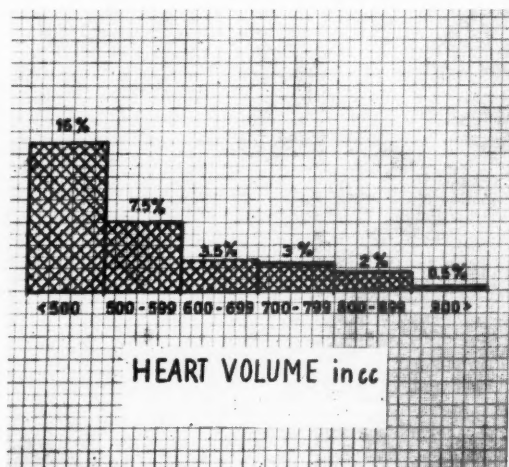


Fig. 1. — If the frequency of prematurity is 5%, the risk of premature birth in the different groups of maternal heart volume will be distributed as above.

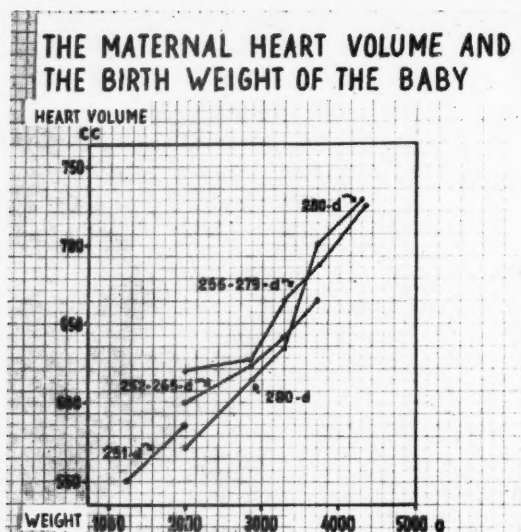


Fig. 2. — There is a good correlation between the weight of the baby and the maternal heart volume the day after parturition, regardless of the duration of pregnancy: < 251 d, 252–265 d, 266–279 d and > 280 d.

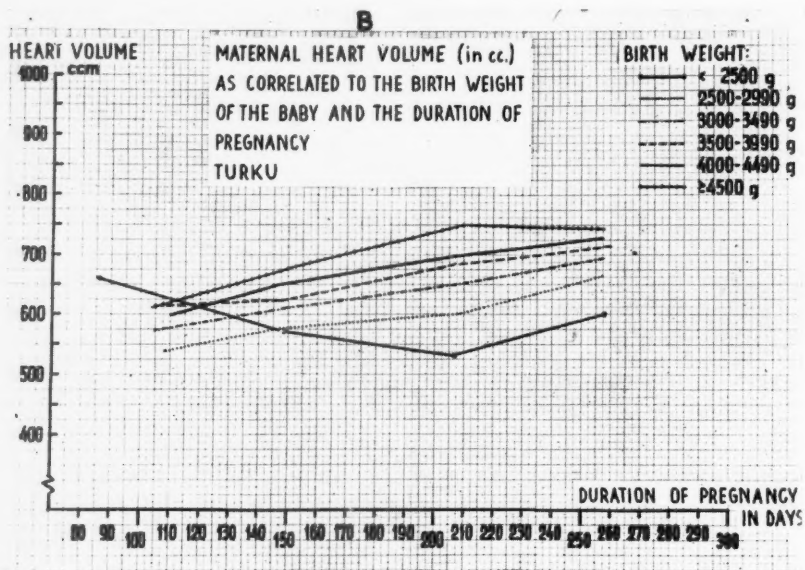


Fig. 3.

This investigation will be continued by increasing the group in which the mothers are ordered to rest and not to carry out heavy work during pregnancy by an addition of all pregnant women who show a decrease in their heart volume during pregnancy.

### **15. An Examination of Premature Children in the Years 1955—1956**

A. MALI, M. KUNNAS and K. VUORINEN  
Helsinki, Finland

On the initiative of the Mannerheim League, and through the mediation of their districts, an examination has been carried out of premature children born alive, altogether 7,905, during the years 1955—1956. The material includes 6,265 children. The tables show:

1. The distribution of premature children according to birthweight.
2. The care of premature children in different hospitals.
3. The mortality of premature children in different age- and weight groups.

Additionally, a short summary is given of the most serious disturbances in development appearing in altogether 4% of the material, and of the social conditions of the families concerned.

### **16. The Results of the Care of Premature Infants at the Aurora Hospital during the Years 1954—1957**

PAAVO HEINIÖ, RUTH WEGELIUS, KERTTU LOUHI-VUORI, MARIANNE BERGROTH  
Helsinki, Finland

The premature baby ward of the Aurora Hospital has 24 beds of which 12 are incubators. Premature babies from the Maternity Departments of Helsinki are treated in the ward. Only babies less than 24 hours old are admitted. Older premature babies are treated in the infant ward of the Aurora Hospital. The babies are brought from the Maternity Departments by the nurses of the Premature ward in the ward's own transportation incubator.

Those babies who need incubator treatment are kept in a temperature between 29°—32°C. The body temperature of the bigger babies is maintained at 35°—36°C, of the smaller ones at 33°—34°C. Partly outdoor-air, and partly oxygen from the oxygen centre are led into the incubators. The oxygen concentration is usually kept at 25—30% and it is controlled every third hour by means of a Beckman's oxymeter. The smaller babies stay in the incubator for

several weeks after the oxygen treatment is interrupted. The baby is discharged when it has reached a weight of 2,500 gr.

The babies are fed almost entirely on breast milk, when necessary by means of an indwelling polythene catheter. On the second day of life, 3—5 gr 5% glucose-solution is given, and on the third day 3—5 gr breastmilk and 3 gr glucose-solution. Babies under 1,250 gr are given skimmed breastmilk. When the body weight reaches 1,500 gr, extra protein in the form of 5 gr cow's milk per feed is added. The breastmilk is mainly brought to the Hospital by the mothers of the babies. If the mothers milk supply is insufficient, the bigger babies get a supplement of 2/3 cows milk and 1/3 water at the age of 2 weeks.

Vitamins: about 3 mg of vitamin K is given during the first day of life. A- and D-vitamins are administered by mouth from the age of 2 weeks. The doses are successively increased until doses of about 5,000 I.U. A-vitamin and about 4,500 I.U. D-vitamin are given daily at an age of 4 weeks. Oil-solutions are used. The administration of C-vitamin starts at the age of 2 weeks, beginning with 25 mg daily and increasing to 50 mg.

The oral administration of iron is started at the age of 3 weeks with small doses which are increased until a daily dose of 70 mg is reached. Thus the baby is used to the full dose of iron, when the iron deficiency of the premature infant sets in. An ironchelate preparation is used ( $\text{Fe}+++$ ).

Preventive antibiotic treatment: During their first days of life, each of the infants gets a penicillin-streptomycin preparation parenterally, 20,000 I.U. penicillin and 12.5 mg streptomycin per kg body weight daily.

Unnecessary handling of the infants is avoided, and unless otherwise necessary, even the physician's first examination takes place only by inspection and following of the temperature, respiration rate and weight charts.

Swabs from the throat, nose and skin are examined for bacteria.

Since the year 1956, ophthalmoscopic examination has been carried out before the infant is discharged from the Hospital.

The health control of the staff is rigorous. Once a month swabs from the nose and throat are examined for bacteria. No masks are used.

An analysis of the results of the premature care is given for the years 1954—57.

The mortality rates were:

Year	Number of infants	Mortality % (total)	Infants' birthweight more than 1,000 gr. Mortality %
1954	81	18.5	17.5
1955	93	19.4	16.9
1956	135	13.3	12.2
1957	162	22.2 <sup>1)</sup>	14.4

<sup>1)</sup> (15 infants' birthweight less than 1,000 gr)

The mortality rates during the first week of life, calculated percentually of the total mortality were:

1954: 86.67 %	1956: 83.33 %
1955: 100.0 %	1957: 94.44 %

Hyaline membrane disease was the main cause of death. This was found in twenty-nine autopsies (33.33%).

This was followed by atelectasis pulmonum, where no other lung finding was evident (13.79%).

Haemorrhagia pulmonum (12.64%).

Infection (12.64%).

Haemorrhagia cerebri (9.20%).

Congenital malformations (3.45%).

Kernicterus (2.30%).

Varia (5.75%).

No post mortem examination was performed in 6 cases (6.90%).

Three cases of retrolental fibroplasia were found during the years 1956—57 in children with a birthweight of less than 1,350 gr.

The mean haemoglobin values were 16.50 gr/100 ml on admission and 9.00 gr/100 ml on discharge from the Hospital during the years 1954—55, when no regular prophylactic iron treatment was given and 10.17 gr/100 ml 1956—57, when prophylactic iron treatment was given.

Sixty-four infants were examined 1—2 months after discharge from the Hospital in the years 1956—57. Their mean Hb-value was 9.30. None of them showed signs of rickets.

## 17. The Growth in Length and Weight of Premature Children during the First Year of Life Taking the Period of Gestation into Consideration

MARJATTA KUNNAS  
Helsinki, Finland

The material includes 392 children, divided in 3 groups according to duration of pregnancy. The differences between the groups are most distinct in the second half-year. It has been stated that the youngest grow most and the eldest, that is to say the full-term children, least.

The growth in length and weight of all premature children is faster than in normal material, excluding the full-term children with a birthweight under 2,500 gr.

### **18. A Case of Panmyelopathy Caused by Chloramphenicol in a Girl One Year and a Half Old**

TUOMAS PELTONEN and SAARA HEIKKILÄ

Turku, Finland

For the treatment of recurrent banal infections, a girl aged one and a half was given «Mixt. Chloramphen, Orion», several times during the course of four months. The total amount of chloramphenicol ingested was 15 grams. The child had become pale and suffered from bleeding mucous membranes, and was therefore sent to the Clinic as a leukemia patient. A severe suppression of hematopoiesis was observed. After the child had been given several blood transfusions and cortisone, her health was restored within six months.

### **19. A Case of Congenital Aplastic Anemia**

SAARA HEIKKILÄ and TUOMAS PELTONEN

Turku, Finland

A two year old girl was admitted to the Clinic because she suffered from anemia. The anemia was diagnosed as being of the aplastic type, but it involved only the erythropoietic system. The body structure of the patient resembled that of a chondrodystrophic. The mental development of the child was normal. The girl was kept alive by means of blood transfusions. Therapeutical measures employed were the administration of ACTH, cortisone, folic acid, cobalt and vitamin B<sub>12</sub> and splenectomy. Following the last mentioned operation, the resistance of the child to infection was appreciably reduced. The last measure undertaken to date has been the transplantation of bone marrow.

### **20. A Case of Juvenile Vitamin B<sub>12</sub> Deficiency**

R. GRÄSBECK and I. KANTERO

Helsinki, Finland

Presentation of a case of B<sub>12</sub> deficiency caused by an unexplained inhibition of the absorption of vitamin B<sub>12</sub> from the gut.

A well-developed boy of 11 years has been treated on numerous occasions since the age of two years for a pernicious-like anaemia. During relapses, changes

in the bone marrow as well as in the peripheral blood are typical of pernicious anaemia. There are no neurological symptoms; the tongue and the spleen are normal. Microbiologically (*L. leichmannii*) determined  $B_{12}$  level in the serum is pathologically low ( $<50 \mu\text{g/ml}$ ). Reticulocytosis and remission after parenteral administration of vitamin  $B_{12}$ . Since the first examination there has always been a slight albuminuria; urography, creatinin clearance, electrophoresis of the serum, non-protein N, etc. have always been normal, however. No macroscopic signs of disturbed gastro-intestinal function, tapeworm-eggs have never been found, there was no improvement after anthelmintic treatment. Radiological examination of the intestines: normal. There is normal fat content in the faeces. Glucose- and starch-tolerance curves are normal. Absorption of  $I^{131}$ -labelled fat: low normal values. The serum  $B_{12}$  binding capacity is normal. No indications were found for the presence of intrinsic factor antibodies in the patient's serum. Parenterally injected radio- $B_{12}$  is retained normally. The gastric juice contains plenty of acid, pepsin, and even intrinsic factor (remarkable increase in the Schilling-value in a classical pernicious anaemia patient). Absorption tests with radio- $B_{12}$  (Schilling-test) are repeatedly pathologically low in spite of the presence of intrinsic factor and are not changed by administration of intrinsic factor, sterilisation of the gut with antibiotics, treatment with cortisone, etc.

Possible causes of the disturbance of absorption of  $B_{12}$  are discussed: the most likely cause is a disturbance in the intestinal wall, possibly a congenital lack of the vitamin  $B_{12}$  acceptor mechanism. This may be combined with an extremely mild steatorrhoea and an absorption anomaly in the kidney tubules.

## 21. The Intestinal Absorption of Orally Administered Trivalent Iron

KAISA LAPINLEIMU and RUTH WEGELIUS  
Helsinki, Finland

It is usually considered that an iron preparation administered by mouth should contain bivalent iron in order to be properly absorbed in the intestines. The authors have carried out iron absorption tests as described by Jasiński with  $\text{Fe}^{++}$  and  $\text{Fe}^{+++}$ . 132 mg  $\text{Fe}^{++}$  (Ferronicum, Sandoz) or 152 mg  $\text{Fe}^{+++}$  (Plexofer, Medica) given by mouth to each of the test subjects, 17 infants and children with iron deficiency anaemia (M.C.H. 13—20, mean value 16, serum iron value 26—102, mean value 75 %, method Kingsley & Getchell). The increase in the serum iron value was to the same extent whichever preparation was employed.

Oral Fe<sup>+++</sup> treatment with Plexofer (Ferric Sodium EDTA) in 15 infants and children suffering from iron deficiency anaemia resulted in a distinct increase in the Hb, M.C.H. and serum iron values in most of the patients.

## 22. Haemophilia in Finland

H. R. NEVANLINNA and E. IKKALA

Helsinki, Finland

The series of haemophiliacs to be presented are collected from hospital records in the whole country, and from data obtained from the Medical Board. It consists of patients who are all living and who have been treated in hospitals since 1930. All patients have been personally investigated by the authors.

The investigation consisted of controlled medical history, clinical investigation as well as of the following laboratory tests:

- bleeding time (Duke)
- Rumpel-Leed test
- platelet count
- coagulation time (Lee-White or Bürker)
- prothrombin consumption test
- thromboplastin generation test (Biggs and Douglas)

The quantitative determination of AHG was performed according to Biggs, Eveling and Richards with small modifications, that of the Christmas factor according to Koller.

In the table, the distribution of the families and patients in A- and B-hemophilia is presented, as well as the distribution according to the degree of severity

DEGREE OF SEVERITY			
	Severe	Moderate	Mild
Hemophilia A .....	64	11	8
Hemophilia B .....	10	10	7
Per cent AHG or F IX	5 severe		
	6—10 moderate		
	11 mild		
NUMBER OF CASES			
	Families	Patients	
Hemophilia A .....	61 (87 per cent)	83 (76 per cent)	
Hemophilia B .....	9 (13 per cent)	27 (24 per cent)	
Total 70		110	

of the disease. At the present time, there are at least 15 additional patients in respect of whom the detailed investigation has not as yet been carried out.

In addition, the authors have knowledge of 6 patients suffering from angiohaemophilia (low AHG with simultaneous long bleeding time) representing four families.

## **24. The Incidence of Leukemia in Finland during the Period 1936—56**

TUOMAS PELTONEN and ANNA-LIISA PYNNÖNEN

Turku, Finland

In 1936—56, 544 children died of leukemia in Finland. The annual death rate due to leukemia has increased fivefold in children during the period in question. The mortality for the whole population has risen from 1.87 to 5.30 per 100,000 inhabitants. The death rate due to this disease is lower in the northern and eastern parts of the country.

## **25. Intimal Changes in Pulmonary Vessels in Congenital Heart Disease in the First Years of Life**

V. ESKELUND, FR. THERKELSEN, I. BOSSEN and J. LIND

Copenhagen, Denmark

Stockholm, Sweden

## **26. Isolated Pulmonary Stenosis before and after Surgery**

D. IKKOS, J. HANSON, C. CRAFOORD and C. O. OVENFORS

Stockholm, Sweden

1. Pulmonary stenosis is a congenital cardiac malformation in which the defect can be localized in:
  - a) the pulmonary valve
  - b) the infundibulum of the right ventricle
  - c) multiple regions of the pulmonary arterial tree
  - d) combinations of the above.
2. The incidence of pulmonary stenosis in 1,000 consecutive cardiac catheterizations was 8%.

3. Pulmonary stenosis has as its consequences:

- a) a harsh systolic murmur with a weak second heart sound over the pulmonary area
- b) increased right ventricular pressure and a gradient between the pre- and post-stenotic areas
- c) right ventricular hypertrophy as seen in
- d) ECG changes and
- e) an abnormal x-ray picture with increased heart volume, bulging right ventricle and decreasing lung vascularity.

4. The types of surgical procedures for correction of the defect can be divided into «blind» and «open» methods.

5. Successful operation results in:

- a) disappearance of or decrease in the intensity of the systolic murmur.
- b) decrease in the right ventricular pressure and abolition of the gradient
- c) decrease in right ventricular hypertrophy as shown by
- d) normalization of the ECG and
- e) decrease in radiological heart volume and disappearance of the valvular dome.

6. Comparison by statistical analysis of the two major operative methods based on the present series of 25 patients and an additional 99 patients presented in published reports has shown a significant difference in the operative results favouring the «open» method.

In addition, it was found that a significant correlation existed between the pre- and post-operative right ventricular pressures, in such a manner that the higher the pre-operative pressure, the less satisfactory might be the postoperative result, regardless of the operative method used.

## **27. Electrocardiographic Criteria for Enlargement of the Right and the Left Ventricle in Children under the Age of Two Years**

BJARNE MERRILD and JOHN LIND

Søborg, Denmark  
Stockholm, Sweden

The electrocardiographic criteria are based on the sizes of the ventricles determined by angiocardiology (and/or general X-ray examination, cardiac catheterization, autopsy) for 130 patients under 2 years with congenital cardio-

vascular anomalies. All with non-enlarged ventricles have been claimed as diagnosed correctly by electrocardiography.

1. Electrocardiographic criteria for enlargement of the right ventricle (+ RVH) of which 3 criteria at least are claimed as being satisfied, and for enlargement of the left ventricle (+ LVH) of which 2 criteria at least are claimed as being satisfied.

	Normal values (mean) in tenths of a Millivolt <sup>1</sup>		Criteria for:	
			+ RVH	+ LVH
Age in Years	0—1	1—10	0—2	0—2
Q <sub>1</sub> .....	1.15	1.11	> 3	
Q <sub>3</sub> .....	3.26	2.50		> 5
QV <sub>s</sub> .....	2.09	1.53	> 3	
R <sub>3</sub> .....	8.91	7.99	> 20	
R <sub>a</sub> VR .....	2.32	1.60	> 6	
R <sub>a</sub> VF .....	8.15	9.30	> 15	
RV <sub>i</sub> .....	13.61	7.15	> 20	
RV <sub>s</sub> .....	14.49	18.97		> 20
S <sub>1</sub> .....	4.34	2.88	> 11	
S <sub>3</sub> .....	2.18	1.70		> 8
SV <sub>i</sub> .....	11.88	7.53	> 26	
SV <sub>s</sub> .....	7.00	3.62	> 20	
T <sub>2</sub> .....	2.84	3.25	> 2.5	
TV <sub>i</sub> .....	2.27	3.41	> 2	
VATV <sub>i</sub> .....			> 0.03 sec.	
VATV <sub>s</sub> .....			> 0.02 sec.	> 0.04 sec.

2. The frequency with which the criteria are satisfied in a series of 130 children under 2 years of age:

After clinical establishment (general X-ray examination, angiocardiography, cardiac catheterization, possibly autopsy) the right ventricle is found to be nonenlarged for 16 patients and enlarged for 114 patients. None of the 16 patients fulfils more than 2 criteria. Of the 114 patients, 47 satisfy at least 3 of the 13 criteria for enlargement of the right ventricle (+ RVH).

After clinical establishment, the left ventricle is found non-enlarged for 47 patients and enlarged for 83 patients. None of the 47 patients satisfies more than one criterion. Of the 83 patients, 16 satisfy at least 2 of the 4 criteria for enlargement of the left ventricle (+ LVH).

3. The sizes of the single ventricles by electrocardiographic and clinical establishment:

The correspondence between clinical and electrocardiographic establishment of the single ventricles is better for the right (47 of 114 patients with enlarged

right ventricle) than for the left ventricle (16 of 83 patients with enlarged left ventricle).

4. The combined sizes of the ventricles by clinical and electrocardiographic establishment:

The correspondence between clinical and electrocardiographic establishment of the combined sizes of the right and the left ventricle is better when only one ventricle is enlarged (12 of 43 patients with enlarged right ventricle and non-enlarged left ventricle and 5 of 12 patients with non-enlarged right ventricle and enlarged left ventricle) than when both ventricles are enlarged simultaneously (8 of 71 patients with enlarged right and left ventricle).

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### 28. Pathology of Congenital Heart Disease

R. LANDTMAN, L. HJELT and E. K. AHVENAINEN  
Helsinki, Finland

From 1948 to 1958, 2,837 autopsies were performed at the Children's Hospital, University of Helsinki. Congenital malformations of the heart were present in 245 instances (8.6 per cent). The incidence of these malformations has gradually increased from 4.8 per cent in 1948 to 14.8 per cent in 1957. One hundred and twenty-five of the children died during the neonatal period (50 per cent) and 229 (93.5 per cent) died within the first year of life. Only five children were over five years old. The birth weight was less than 2,500 g in 80 cases (32.7 per cent). Malformations of other organs, often multiple, were present in 105 cases (42.9 per cent). The organs most frequently involved were: urinary tract 46, gastrointestinal tract 40, skeleton 38, central nervous system 24 and respiratory organs twelve cases. The most common immediate causes of death were: pulmonary complications (172), intracranial hemorrhage (41), and gastroenteritis (48).

Detailed records were kept of the clinical data of the patients and of the autopsy findings. The acyanotic group comprised 120 cases of which the most common were: ventricular septal defect (25), auricular septal defect (20), coarctation of the aorta with or without patent ductus arteriosus (18), patent ductus arteriosus (7) and others (50). One hundred and twenty-five children belonged to the cyanotic group (transposition of the great vessels 44, truncus arteriosus thirtythree, bi- or trilocular heart 22, tetralogy of Fallot 9, and other 11).

*Microscopic examinations: Lungs.* Changes in the pulmonary vascular bed were observed in 226 (92.2 per cent) of the cases, 108 of which belonged to the cyanotic group. Arteriosclerotic changes (41 cases), which were mainly confined to the small arteries and arterioles, consisted of intimal proliferation, medial hypertrophy and thickening of the adventitia. Arteritic changes occurred in 106, compensatory changes in 103, and vascular anomalies in 10 instances. In addition, biopsies were obtained from 43 children with patent ductus arteriosus at the operation. Changes in the pulmonary vascular bed were observed in all these specimens. There was no positive correlation between these changes and the pressure in the pulmonary artery obtained at cardiac catheterization. *Liver.* Fat infiltration was present in 141 cases (57.5 per cent), 67 of which belonged to the acyanotic group. Centrilobular necroses were seen in 12 instances. The aforementioned histological changes were illustrated by microphotographs. The studies, which will also include other organs, are in progress.

## **29. Cardiovascular Effects of Adrenaline, Noradrenaline, Acetylcholine and Histamine in Newborn and Young Pigs**

LEO HIRVONEN, JOHN LIND and TUOMAS PELTONEN

Turku, Finland

Stockholm, Sweden

Increasing doses (1–20  $\mu\text{g/kg}$  b.w.) of adrenaline, noradrenaline, acetylcholine and histamine were injected intravenously into ten newborn pigs (weighing 1.1–1.7 kg) and five 2–3-week-old pigs (3.3–4.5 kg). The aortic and right ventricular pressures were recorded simultaneously by means of two Sanborn manometers.

Adrenaline and noradrenaline increased, and acetylcholine decreased both the aortic and the right ventricular pressures in both pig groups.

After histamine injections, the aortic pressure of the older pigs decreased markedly. With the highest doses the fall in systolic pressure was 30 to 60 per cent. The changes in aortic pressure of the newborn pigs were variable. A decrease, an increase, or no change was found in these animals. The right ventricular pressure underwent no significant changes either in the newborn or in the older group.

### 30. Vascular Complications in Coarctation of the Aorta

B. LANDTMAN and LEENA TUUTERI

Helsinki, Finland

Vascular complications were seen in 4 of 68 children, with coarctation of the aorta. In 3 of the cases the complications occurred after surgical correction of the coarctation. The complications were: Intracranial hemorrhage 2 cases, rupture of intercostal artery into the esophagus 1 case, and mesenterial thrombosis followed by intestinal necrosis. Three of these 4 patients died.

### 31. Circulatory Studies in Patent Ductus Arteriosus

ERIC IVAR WALLGREN

Helsinki, Finland

Cardiac catheterization and determination of the «true endogenous creatinine clearance» and para-amino-hippuric acid clearance were carried out pre-operatively on 20 children with patent ductus arteriosus. A decreased renal plasma flow was demonstrated in children with a big shunt through the patent ductus. The ratio of endogenous creatinine clearance to renal plasma flow was increased if the left-to-right shunt was above 40 per cent, even in the presence of a normal pressure in the pulmonary artery.

### 32. Operative Treatment of Vesico-Ureteral Reflux

MOGENS ANDREASSEN, AUGUST HALBORG SØRENSEN, JOHN LINDENBERG,

HENNING ANDERSEN

Copenhagen, Denmark

It seems to be possible to correct the vesico-ureteral reflux in a number of ways.

In the HUTCH-operation (J. Urol. 68:457, 1952) the normal entrance of the ureter into the bladder is preserved, and the lower end of the ureter is placed inside the bladder; during distension of the bladder this part of the ureter is compressed and the reflux is thus stopped.

The lower end of the ureter is cut off and the ostium is closed. Through an incision in the mucosa of the bladder, a curved forceps is forced in an oblique

direction through the bladder wall. The ureter is placed into this oblique canal, partly getting a course through the muscles of the bladder wall, but mainly a submucosal course before the entrance into the bladder.

The end of the ureter is sutured (mucosa—mucosa) to the inside of the bladder. Finally the external wall of the bladder is sutured around the ureter. During distension of the bladder, the intra-vesical part of the ureter will be compressed mainly by the «mucosa pressure» and partly by the muscular tension.

Five children have so far been treated with this operation. In 2 cases out of seven operations, 2 recurrences occurred and were re-operated.

In the cases with no recurrence the patient's condition is good.

### 33. A Comparison between the Results Obtained by Urography and Mictiocyctography in Pediatric Urological Disease

P-E. HEIKEL and K. V. PARKKULAINEN

Helsinki, Finland

Mictiocyctography (MC) has proved to give valuable information, even about the upper urinary tract, and consequently there is some justification for a comparison between the results obtained by excretory urography and MC. Both examinations were carried out on 79 patients in the Children's Clinic, University of Helsinki, during the years 1955—56. The MC technique was: the bladder was filled by a catheter with a sterile suspension of 25 per cent colloidal bariumsulphate (Collobar, Astra) until a strong voiding reflex was noted. The suspension does not irritate the bladder and no anaesthesia is needed. Radiograms were made of the filled bladder and possible ureteral reflux. The child was then turned so as to lie on its side and as many radiograms as possible made during micturition. A further radiogram was made after the end of micturition to show possible vesical residual and ureteral reflux.

The results are shown in the table:

				Urography findings	
				normal	pathological
MC-findings	pathological	53		25	28
	normal	26		20	6
Totals		79		45	34

In the cases where the results obtained by both methods were pathological, on 19 occasions the MC findings gave quite sufficient information, even about the upper urinary tract, whereas in only 9 cases did the urography reveal facts of interest beyond the MC findings. When the changes in toto are situated proximal to the vesicoureteral junction, no ureteral reflux is possible and in these cases the urography is of course the only method of demonstrating the site of the changes; but of 59 pathological cases only 6 belonged to this group. The MC is also superior to the passive, retrograde urethrography.

Urography was superior to the MC findings in 15 cases of 79.

Both methods were of equal importance in 30 cases of 79.

The MC findings were superior to the urography, in which the findings were insufficient in 34 cases of 53.

To summarize the results, it can be stated that MC is a very valuable urological roentgenological examination in childhood. The main indications are a) urinary infections of long standing or of recurrent type, b) when residual urine is found, c) in micturition disorders, d) in cases with congenital malformation of the lumbosacral spine or of the external genitals and e) in cases with enuresis of long standing. Owing to the radiation hazards to the gonads the MC may be carried out on indications other than those here mentioned only after special consideration.

### 34. Treatment of Childhood Nephrosis

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Sixtyfive cases of nephrotic syndrome, treated in 1947—1956 at the Children's Hospital, Helsinki, have been reviewed. Of these 61 could be traced. 23 were dead. In 28 cases, a follow-up examination was made, in ten cases information about the child's health was obtained by means of a detailed questionnaire. Five children are still under treatment.

The observation period after the onset of edema in all cases was more than one year and a half, and in 23 cases more than five years.

The material was divided into three groups according to the treatment employed. The first group consisted of 21 children with no hormone therapy, »no treatment group», the second one of 26 children who had received one or more short courses of ACTH and/or cortisone, »short term group». The third group included 14 patients who had been treated by intermittent steroid therapy for 9—15 months, »long term group». The results in the different groups are shown in Fig. 1.

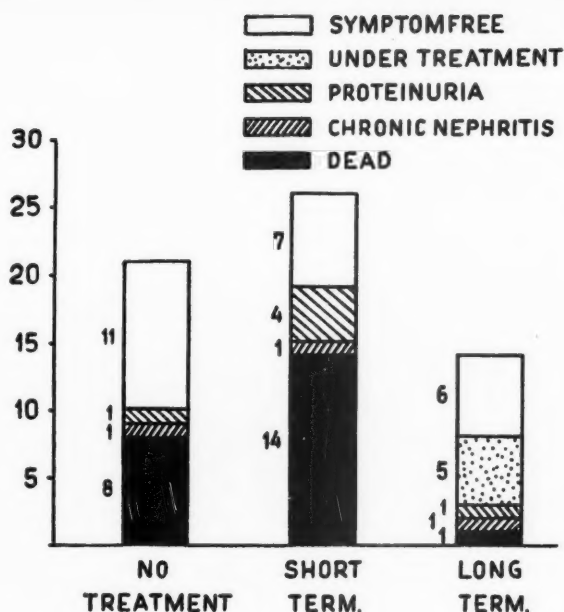


Fig. 1.

The mortality during the first five years of the disease was significantly lower in the «long term group» than in the two others. Though it is not yet possible to draw final conclusions about the influence of the long term therapy on the ultimate prognosis of nephrosis, it seems to prolong the life of these children.

This work will be published in detail elsewhere.

### 35. Congenital Deficiency of the Abdominal Muscles; A Series of Eleven Cases

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Helsinki, Finland

The age of the patients varied from three days to ten years. Ten were males, one was female.

The syndrome consisted of the following components:

- 1) Deficiency of the muscles of the anterior abdominal wall of varying degree.

The loss of muscular tissue was most severe in the lateral muscles. The upper portions of the rectus muscles were least affected. The right side was always more affected than the left.

2) Urological anomalies in all cases sufficiently examined. The picture was that of lower urinary tract obstruction with residual urine, vesicoureteral reflux into the dilated tortuous ureters and kidney damage. There was patent urachus in three cases. It is suggested that the cause of obstruction is the sharp forward angulation of the bladder due to lack of support from the abdominal muscles.

3) Non-descent of the testes in nine out of ten males.

4) Various other anomalies: Pigeon breast, scoliosis, arthrogryposis, cardiovascular anomalies etc.

Treatment consisted of cystostomy for three patients, nephrostomy for one, bladder neck resection for one and plastic operation of the abdominal wall for two.

Six patients died between the ages of nine and thirty-six days, one at three months, one at seven years. Two males, aged nine and ten years, and the only female, aged one year, are living at the time of this report.

### 36. The Treatment of Enuresis Nocturna with Conditioning Devices

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In this study, we have employed two different devices (Crosby: Med. J. Austral. 1950, p. 533 and Davidson & Douglass: Brit. Med. J. 1950, vol. I, p. 1345). The results seem to be independent of the type.

The aim of the study has been:

- 1) To test the effect of the treatment upon the enuresis.
- 2) To investigate if any harmful or favourable psychological effect of the treatment can be demonstrated.
- 3) To investigate if the psychological examination or other factors permit a proper selection of children for treatment.

We have treated 21 children aged five to 17 years. Four of these children have had »dry» periods of long duration. This condition is named »secondary» enuresis. We have not treated children with organic diseases in the urinary tract or in the nervous system, epilepsy, oligophrenia, present or former encopresis or grave emotional disturbances. Otherwise the material is not selected. The children were examined before treatment and half a year after treatment. Both examinations have included personal interviews with child and parents, Rorschach test and Children's or Thematic Apperception test or Düss test. The first

examination has also included an intelligence test (Binet-Simon or Raven's Progressive Matrices, 1947).

*Results:* ad 1) 12 children were dry every night at the time of the follow-up examination and three almost every night (one or two wet beds a month). Six children were unchanged or only slightly better. There were three relapses after more than half a year: in a girl in connection with a gonorrhoea with urethritis, in a boy after a traffic accident with cerebral concussion, and in another boy during a conflict in the family. These children have not been treated again.

ad 2) The general psychological condition was unchanged in eight cases. In nine cases it was better, and in four cases worse than before treatment. These four children had primary enuresis. Three of them were six to eight years old and thus in the period of adjustment to school. The fourth child was in puberty. They were all described as more inhibited. The inhibition did not exceed a physiologic degree. The adjustment to the environment was unchanged in eleven, better in eight and worse in two cases. No case of pathologic emotional disturbance was observed.

ad 3) We have investigated the relation between the result of treatment and the following factors: Familiar disposition, type of enuresis (primary or secondary), sex, age, emotional immaturity, I.Q., and type of personality (extrovert or introvert). None of these factors permits a selection of children for treatment as the results are good in all groups, although it seems that female sex and emotional immaturity decrease the chance of success.

Like other investigators we have found a preponderance of emotional immaturity among children with enuresis. Fifteen of the 21 children were characterized as emotionally immature.

### **37. Psychosomatic Studies of Children with Congenital Heart Disease**

B. LANDTMAN and E. VALANNE  
Helsinki, Finland

Eighty-three children with congenital heart disease were studied. The age of the children varied from 5 to 15 years. The authors were assisted by a team comprising a child psychologist, a social worker and ward personnel.

Detailed information of the development and psychosomatic pattern of behaviour was obtained from the parents prior to surgical correction of the cardiac malformation. Each of the patients underwent a thorough psychoso-

matic examination in the hospital. Follow-up examinations were carried out 3 and 12 months after the operation. The final evaluation of each case took place in the hospital in the same way as before the operation.

Till May 1958, 42 of the children had been in the hospital for final evaluation. All social classes and family backgrounds were represented. The heart disease had been detected in infancy in 14 cases and in 15 cases after the fourth year. The majority of the children had a patent ductus arteriosus. The exercise tolerance was normal in 19 instances; the remainder of the children suffered from dyspnea on effort. Following surgery, the exercise tolerance was normal in 35 cases. Prior to operation, 27 of the children suffered from repeated upper respiratory infections but only 6 after the operation. The physical condition (weight and length) of the children improved considerably in most of the cases after the operation. X-ray examination revealed cardiac enlargement in 22 cases before and in 10 cases after the operation. The corresponding figures for electrocardiographic signs of hypertrophy or strain were 32 and 14.

According to the mothers, the pattern of behaviour of the children at home was normal in 21 cases. Twenty-one children showed symptoms of severe maladjustment (enuresis, encopresis etc.). Following operation an improvement occurred in 17 cases. The attitude of the mother towards the children was considered normal in 24 and overprotective in 18 cases.

The mean IQ value somewhat increased after the operation in the different age groups.

The sleep was poor or disturbed in 24 cases before and in 9 cases after the operation. The appetite, which was poor or fair in 25 cases before the operation, improved in 17 cases following surgery.

Only one child was left with a negative impression of the operation and the hospitalization.

### **38. Congenital Hyperpituitarism of Hypothalamic Origin; A new Diencephalic Syndrome with Endocrine Manifestations**

M. SEIP

Oslo, Norway

Three patients, two of whom are brother and sister, are presented. From early infancy they showed the following main features: Acromegaloid gigantism, extreme waste of subcutaneous fat, hyperlipemia, hepatosplenomegalia with fatty infiltration of the liver and spleen, cirrhotic liver changes, abnormal carbohydrate metabolism, general muscular hypertrophy with increased muscular glycogen, hypertrichosis, hyperpigmentation, punctuate cornea.

capacities, advanced bone age and dental development, and perhaps slight cardiomegaly (glycogen deposits?) and hypertension. The results of the most important laboratory studies in these patients are presented.

The clinical features and laboratory data are explained by an increased formation of several anterior pituitary hormones, growth hormone, ACTH, MSH, the adipokinetic hormone (adipokinin). Most of the signs and symptoms are thought to be caused by hypersomatotropinism. The clinical observations, however, favor the concept that an adipokinetic hormone, distinct from growth hormone, is produced by the hypophysis, a concept which is still under debate.

Pneumoencephalographic studies revealed dilatation of the third ventricle in two of the reported cases and of the basal cisterns in all three, pointing to a hypothalamic lesion. The hyperpituitarism is thought to be due to this hypothalamic lesion. In the two cases belonging to the same family a hereditary malformation of the diencephalon is thought to be present (possibly recessive inheritance). In this family the parents were second cousins. Both parents and one brother showed definite hyperlipemia with a similar pattern as our patients, but to a lesser degree, and other signs of the disease were lacking.

Two similar cases have been reported from Brazil by Berardinelli in 1954. However, he did not give his attention to the diencephalon as the primary site of the disease.

### 39. Retention of the Thyroid Gland

HENNING ANDERSEN  
Copenhagen, Denmark

A comparison is made of two groups of hypothyroid children, one with no functioning thyroid tissue anywhere, and one group with small amounts of sublingual retained or aberrant tissue as the only source of activity localized and estimated by  $I^{131}$  (from 4—15% uptake). In patients under treatment this was stopped at least one month prior to the uptake study. Both conditions were considered to be fetal in origin, and the purpose of the study was to compare the outcome of the two groups which accidentally comprised 9 patients in each group.

In the athyrotic group the diagnose was made in 6 cases within the first three months, in two cases before 6 months of age, and in 1 case (eskimo) the moment of time could only be fixed as «under one year».

In the retention cases two children were diagnosed at the age of 4 and 7 months — the rest were diagnosed at times from 2 to 7 years of age. The times of diagnosis in this group roughly corresponded to the amounts of functioning tissue found.

The mental outcome was that in the athyrotic group 2 were mentally retarded, the rest were feeble-minded. In the retention group, 6 were normal, one was mentally retarded and one probably normal, diagnosis at the age of 4 and 7 months, respectively.

The children have been treated from the time of diagnosis — thus the treatment has been on the average of longest duration in the athyrotic group.

It is not possible from the measurements of thyroid function in the retention group to draw a definite conclusion about the activity of the fetal thyroids, but one may assume that it has been subnormal. However, it has been sufficient to maintain the children euthyroid for a shorter or longer period after birth.

It is tempting to assume, that the amount of sublingual tissue present in fetal life accounts for the better mental outlook in this group. Except for one child who died from toxoplasmosis, no signs of brain damage coinciding with the athyrosis could be demonstrated, but for instance enzymatic defects could not be definitely excluded.

#### 40. Fetal Thyroid Function

HENNING ANDERSEN, HILDE LEVI and JOHN LIND  
Copenhagen, Denmark and Stockholm, Sweden

To 29 gravida admitted for legal abortion, 30 micro-Curie of  $I^{131}$  was given at various intervals before the evacuation was performed. The fetal thyroids were dissected and their radioactivity estimated by a scintillation counter, and autoradiograms made.

Activity could be demonstrated from fetuses of approximately 10 cm in length (crown-rump), corresponding to 12—14 weeks of gestation and at a time when thyroid follicles were beginning to differentiate. This corresponds to previous findings made by other investigators.

During the 12th—25th weeks of gestation examination showed a steep and gradual increase in the thyroid activity.

The relation, fetal thyroid activity to fetal length and weight, was similar.

The total amount of  $I^{131}$  taken up by the fetal thyroid increased from 0 to about 2% of the dose given.

There was a good correlation between the activity as measured by the scintillation counter and that estimated by the autoradiograms.

The interval between maternal intake of  $I^{131}$  and the time of evacuation i.e. the time for fetal thyroid iodine uptake, did not, within rough limits, seem to, have much influence upon the amount of uptake. This was confirmed by

selecting groups of fetuses of approximately equal weights and different uptake times. One such group consisted of 9 fetuses weighing from 205—250 grams. Another group consisted of 4 fetuses weighing 250—280 grams. In these groups, it could be demonstrated that after approximately 6 hours an equilibrium was reached, after which the activity in the thyroid was constant. From infancy and onwards, this state of equilibrium is not reached until after 20—24 hours, which may indicate that the fetal thyroid has a very rapid iodine uptake.

#### 41. Dental Changes in Some Endocrine Disorders

HENNING ANDERSEN and GRETHE HOLST  
Copenhagen, Denmark

Examination was made of 19 children, aged 2—16 years, with *hypothyroidism*. In 11 untreated or insufficiently treated cases, the bone development (Greulich's Standard) was more retarded than the dental development. In 5 sufficiently treated cases they were identical, whereas in 3 extensively treated cases the bone age was more advanced than the dental age. Additionally, 5 cases of *idiopathic pubertas precox* were examined and they all showed a more advanced bone age than dental age. In the conditions mentioned, bone tissue seems to be more sensitive to variations in hormone (thyroid and sexhormones) secretion than does dental tissue.

Dysmineralisation was found in a remarkably high percentage of cases (80% compared with the «normal» 5—18%) and in most cases indicated intra-uterine dental disturbances. This may be due to lack of thyroid hormone during intrauterine life, and compares with bone retardation at birth seen in athyreotic children.

Hypoplasia of the jaws, especially the mandibula, retarded formation of the dentin and signs of macroglossic pressure were frequent findings.

The percentage of *caries*, often reported as high in this disease, was 37% of 390 teeth examined (temporary and permanent) and well within «normal» limits (26—49%).

The size, shape and number of the teeth were normal.

Case reports are shown on the dental changes in *hypoparathyroidism*, and in a peculiar case of *hyperthyroidism* in a boy with *mongolism*. In this patient the dental development showed signs of acceleration (hyperthyroid?) together with retardation and malformation (mongolism?).

In addition, the dental status of two patients with «non-endocrine» disorders are shown, one with *ectodermal dysplasia*, the other with *chondroectodermal dysplasia* (Ellis-vanCrefeldt).

## 42. Treatment of Diabetes in Children with Bz-55

GUNNAR ENGLESON and ORLA LEHMANN  
Lund, Sweden

Reports in publications of the results that have been obtained with sulphonyurea derivatives in treating certain forms of diabetes of the aged are fairly unanimous, but the information on the effect of the preparation against juvenile diabetes varies somewhat. Little has been written about peroral therapy for diabetes in childhood and most authors are of the opinion that no definite effect is obtained with these preparations and that they are to be regarded as contraindicated for this form of diabetes.

In our experiments we employed Bz-55 in doses of from 4 to 7 g daily during the first days of therapy, aiming for an Invenol concentration as high as 60–70 mg%, after which the dosage was slowly reduced.

The material consisted of 12 diabetics aged 4–12, 5 girls and 7 boys. Of these, eight were newly discovered and 6 had not previously been treated with insulin. The duration of therapy was 3–8 months in 3 cases, in the others only 1–2 weeks. The therapeutic experiments were initiated at the beginning of 1955, starting with Invenol and dietary regimen only and gradually moving to a combination of insulin-Invenol and diet.

*Results:* Of the 8 patients who had been given combined therapy, 4 were newly discovered. It was possible in these cases to establish after the termination of treatment an exceptionally low insulin requirement for the age, less than 10 international units in all the cases. In 3 cases previously insulin-treated we observed only a slight decrease in the insulin requirement. One patient deteriorated rapidly under Invenol alone and entered into a pre-coma after 1–2 days. Two cases which it was possible to treat solely with Bz-55 for a longer period showed gradually distinct signs of insulin deficiency, retarded growth and a poor general condition. It became necessary to switch to insulin. The therapeutic results were unsatisfactory for 2 girls at the age of puberty.

With the large doses of Bz-55 employed, there was a fairly high incidence of side-effects in the form of exanthema, fever and slight leukopenia despite prophylactic antihistamine therapy. In a number of cases these conditions led to discontinuation of the oral therapy. D 860 has also been tried and, administered in fairly large doses, has given similar results to Bz-55.

We came to the summary conclusion that peroral treatment of diabetes in children can only be considered for new cases and then in combination with insulin and a dietary regimen. Long-term therapy—even combined with insulin—is not advisable in our experience. Peroral treatment for juvenile diabetes in general clinical practice is not advisable.

### 43. Tolbutamide in Juvenile Diabetes

YNGVE LARSSON  
Stockholm, Sweden

1. Sulphonylurea therapy with tolbutamide was tested in 33 fresh cases of juvenile diabetes, 15 boys and 18 girls, from 1 to 16 years old.
2. Tolbutamide alone *in the initial phase of diabetes, without concomitant insulin* (2 cases) produced no discernible effect on the levels of sugar in blood or urine.
3. When tolbutamide administration was begun *in a postinitial phase*, when the patient had received insulin for some months (11 cases) a clear normalizing effect appeared in 2 cases, but ceased after treatment had been given for 1 and 4 months, respectively.
4. When tolbutamide was given *in an early initial phase, concurrently with insulin*, and the diet was moderately restricted (20 cases), a definite effect was seen in 18 cases. This effect, which is still present in 15 cases, took the form of greatly reduced insulin requirements: for long periods the patients required no exogenous insulin and yet showed normal blood sugar levels and absence of glycosuria.
5. In a case of prediabetes a diabetic *glucose tolerance normalized* in association with tolbutamide therapy.
6. The negative attitude which earlier writers have displayed to sulphonylurea compounds in juvenile diabetes is unwarranted. Under the conditions outlined here, such drugs can produce a *remission in the postinitial phase of diabetes*. Even if its effect proves to be transitory, early treatment in this form may favourably influence the continued course of the disease.
7. These findings support the theory of a direct *pancreatotropic, beta-cell stimulating effect* of tolbutamide. In the successful cases it may be assumed that remnants of islet tissue were stimulated to increased insulin secretion after they had been protected by exogenous insulin against deleterious hyperglycaemia.

### 44. The Height and Weight Development in Diabetic Children

O. SOMERSALO, H. HIEKKALA, L. TUUTERI and P. RANTAKALLIO  
Helsinki, Finland

The material consists of 317 children, 178 boys and 139 girls. They were treated at the Children's Hospital, Helsinki, during the period 1947—1956. Their height and weight development have been compared with normal investigation material.

At the onset of the disease, the height and weight of these children did not differ from the normal material. In the group of diabetics who have had the disease for more than 6 years a slight retardation in the height development can be seen. In the weight curves there are no clear differences. However, among the short children there are many patients who are overweight and have hepatomegaly.

The diabetics are divided into three groups according to the daily sugar output.:

1. sugar output less than 30 g/day
2. sugar output 30—60 g/day
3. sugar output more than 60 g/day.

No significant differences from the normal controls can be seen in any of these groups.

The physical development of the diabetics was also investigated according to the occurrence of ketosis. No variations were found in the children belonging to the groups »no ketosis» and »occasionally ketosis». A slight tendency towards growth retardation can be noted in the children in the group »often ketosis».

#### **45. Periosteal Thickenings in Childhood**

P.-E. HEIKEL and S.-L. KOSKELA

Helsinki, Finland

Three cases are presented: two twin boys and a girl, all a little over one year old and from extremely poor social circumstances. There was no evidence of either hyper- or hypovitaminoses or of infection. The bone thickenings were periosteal, and not cortical as in Coffey's disease, and neither was their localisation typical of that disease. The children recovered after some months without specific therapy. The diagnosis is open.

#### **46. A Case of Deforming Osteochondrodystrophy**

SAARA HEIKKILÄ and TUOMAS PELTONEN

Turku, Finland

An one month old boy (with a weight of 3,200 g) was brought to the Clinic, where the child was seen to have abnormally short forearms and legs. The upper part of the external ear was also deformed. The child's weight had

dropped to 2,000 g. The child was later examined several times during eight years. He was mentally underdeveloped, his I.Q. was 75, and he was aggressive. Deformities had gradually developed in the long bones and in the discs of the spinal column. The base of the skull was short. At the age of seven and a half years he was 101 cm long and weighed 17.3 kg. A cousin of the child's paternal grandfather was only 155 cm long when fullgrown.

#### 47. Subluxatio Capituli Radii in Children

O. SNELLMAN

Helsinki, Finland

Subluxation of the head of the radius is described, and it is emphasized that this is the most common lesion of the bones and joints in children under 5 years. As a result of traction, the head of the radius slips in the distal direction under the annular ligament, causing a painful condition without the simultaneous occurrence of fracture or luxation of the elbow. Patients with this type of lesion are most frequently seen by surgeons, but the lesion is not uncommon in pediatric practice, either. The symptoms are few: a sudden pain and slightly limited extension and flexion. There is no swelling over the capitulum and X-rays are negative.

The diagnosis is verified by reposition, which does not need anesthesia. — A series of 474 cases is presented, consisting of 190 boys and 284 girls. The majority of patients were between 1 and 3 years old.

(To be published in the *Acta Orthopædica Scandinavica* 1959.)

#### 48. Nivergelt Syndrome and Its Treatment

K. SOLONEN and M. SULAMAA

Helsinki, Finland

The presented case, a boy 2.5 years of age, with congenital malformed forearms and legs, is similar to the only published case of Nivergelt. In this case, however, familiar inheritance could not be traced. Because of the severe valgus-position, walking was not possible. Both the tibia and the fibula were rhomboid and the epiphyseal lines crossed rectangularly. An osteotomy and wedge resection brought the epiphyseal lines to a nearly normal direction, and the boy became able to stand and walk.

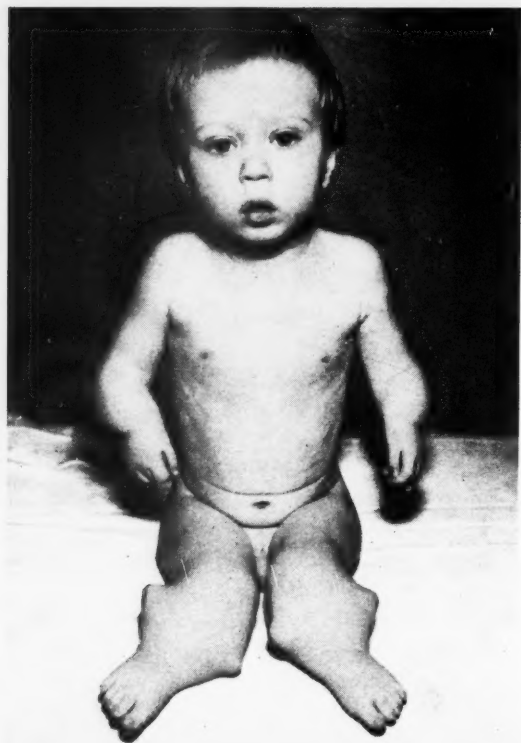


Fig. 1.



Fig. 2.

## 49. Premature Synostosis of the Cranial Sutures

M. SULAMAA and L. LAITINEN

Helsinki, Finland

Because of premature fusion of the cranial sutures, the expansion and development of the normal brain of the child will often be disturbed.

Grotesque deformities of the skull develop. The intracranial pressure increases, headaches, vomiting and convulsions follow. Papiledema leads to optic atrophy and impaired vision, and there will be retarded physical and mental development.

At the Children's Clinic in Helsinki, 60 cases of craniosynostosis have been operated on. A modified linear craniotomy was carried out, the bone margins being covered with polyethylene film. The results of the operation were good. The cranial deformity became less severe or was wholly corrected. The papiledema, headaches, vomiting and convulsions disappeared. The vision, the physical and the mental development improved.

The authors emphasize the importance of early operation.

The typical forms of craniosynostosis were presented in pictures.

## 50. Acute Bacterial Meningitis in Children

JYRKI KAHTIO, MARIANNE PAATELA and ILARI RANTASALO

Helsinki, Finland

During the twelve-year period 1946—57, altogether 379 infants and children with acute bacterial meningitis were treated at the Children's Hospital, University of Helsinki, and the municipal Aurora Hospital.

The bacteriological diagnosis was established in 84% of the cases. *H. influenzae* was the causative organism in 29%, *N. meningitidis* in 21%, and *Str. pneumoniae* in 20% of the cases. Some other bacteria were found in fourteen per cent of the cases.

One fourth of the patients were less than 3 months of age at the onset of the illness. Two thirds became ill during their first year of life. The unreduced mortality was 29% in the whole series. It was highest in the younger age groups.

Around 40% of the fatalities occurred during the first 24 hours in hospital. It was felt that the first measures of treatment should be directed to the alleviation and prevention of the circulatory collapse often present on admission, brain anoxemia and other symptoms of shock.

During the six-year period 1952-57 there was no mortality after 24 hours at hospital in the group caused by *N. meningitidis*. In the cases caused by *H. influenzae* and treated with chloramphenicol this reduced mortality was 6%. In the *Str. pneumoniae* group the mortality was 11% when large doses of penicillin were used. In the heterogeneous group of bacteria other than those mentioned above, the mortality was dependent upon the nature of causative organism and varied within wide limits.

### 51. The Effect of Whooping Cough Vaccine on the Incidence of Whooping Cough during an Epidemic in Helsinki in 1957

MIKKO HIRVENSALO and NILO HALLMAN  
Helsinki, Finland

The material consists of 255 cases which came to the knowledge of the child welfare centres, and 182 hospitalised cases of which 82 were treated at the Children's Clinic and 100 at the Aurora Hospital in Helsinki.

Children who had been given Per-Dif vaccine at least twice at the maximum interval of 3 months were regarded as vaccinated.

In the welfare centre material about a half were vaccinated children. Whooping cough morbidity was c. three times greater among non-vaccinated than among vaccinated children.

The disease was of longer duration and more serious among the non-vaccinated. In these cases the disease lasted for over 6 weeks in 73 per cent, while the corresponding figure was 44 per cent for the vaccinated cases. The welfare centre material included 7 cases of pneumonia among 130 non-vaccinated children while not a single case occurred among the 125 vaccinated cases.

Of the children treated in hospital, c. 9/10 were non-vaccinated. Pneumonia occurred in 45 per cent of the cases, almost exclusively among the non-vaccinated children.

It may consequently be stated that although vaccination against whooping cough does not provide absolute protection against the disease its beneficial effect is nevertheless fairly evident.

### 52. Torsion of the Stomach as a Cause of Vomiting in Infancy

SIGURD EEK and HENRIK HAGELSTEEN

Oslo, Norway

Torsion of the stomach as a cause of vomiting in infancy is common. A collection of gas raises the transverse colon to a position between the liver and the anterior abdominal wall, and the colon, being attached to the greater curvature of the stomach through the gastrocolic ligament, twists the stomach forwards and upwards. The radiological findings are strikingly alike in all cases. Torsion is best displayed when the patient is examined by X-ray in the erect position. He is placed in a special cotton garment, suspended by four straps, which at the same time hold the head erect.

The chief symptom is vomiting, sometimes projectile, beginning soon after birth.

Treatment is simple: the baby is placed either prone or on its right side.

### 53. Follow-up Examination of Children with Hypercalcemia

N. HALLMAN, H. HIEKKALA and A-L. RINNE

Helsinki, Finland

Thirteen cases of hypercalcemia were treated at the Children's Hospital, Helsinki, during the period 1952—1956. At the onset of the disease they were six to twelve months of age. All of them had received vitamin D in abundance. Two patients died in the acute stage of the disease, and one three years later.

We have been able to follow 8 of the remaining 10 cases for 3 to 6 years. The period of hypercalcemia varied from 6 to 18 months. At the present time they all have normal plasma calcium as well as phosphorus and phosphatase values. Urea N was high in all cases at the beginning and 4 show still elevated values.

The growth has been slow in all these patients. The bone age that was earlier retarded is now normal. The mental development was more or less retarded in all cases during the first year of the disease. Two of them now have a normal IQ, four are slightly and two severely mentally retarded.

Calcifications in muscles and lungs were found in seven cases. Three of them are dead, and in four cases the calcifications have disappeared. Osteosclerosis was found in all cases, and it is still present in two patients who have developed a craniostenosis (the same two are severely mentally retarded). One girl has had nephrolithiasis and one boy cornea calcifications for three years.

## 54. Two Cases of Idiopathic Hemosiderosis of the Lung

K. ÖSTERLUND and P-E. HEIKEL

Helsinki, Finland

Two cases of idiopathic hemosiderosis of the lung have been diagnosed in the Children's Clinic, Helsinki. The diagnosis has been made on the basis of the clinical, roentgenological and biopsy findings.

Case 1. Boy, born 1954. He had been pale since birth and often afflicted by respiratory infections. At the first admission to the Clinic at the age of 1 year and 2 months, the roentgenological findings were typical of idiopathic hemosiderosis of the lung. During the following two years he had repeated attacks of bleeding into the pulmonary parenchyma, attended by a lowered general condition, anemia and respiratory distress at every attack. Macrophages containing hemosiderine were found by gastric lavage. During deltacortisone therapy of about one years duration the condition was somewhat improved. In april 1956 splenectomy was performed. Immediately thereafter, he had a pneumonia but the condition was then unchanged compared with the pre-operative state. Six months later, a new attack of severe bleeding into the pulmonary parenchyma suddenly ended fatally. The diagnosis was verified by autopsy.

Case 2. Boy, born 1948. At the age of 3 years he was found to be anemic and suffering from respiratory infection. The anemia then went on unchanged, and was supposed to be due to alimentary factors until the roentgenological findings at the age of 6 years seemed to indicate hemosiderosis of the lung. The diagnosis was verified by gastric lavage. In spite of signs of increasing pulmonary fibrosis, the course of the disease has since then been moderately improved and the boy is still alive in rather good condition.

Two series of chest radiograms are presented showing the rapid variations of the roentgenological chest findings.

## 55. An Apparatus for Continuous Drop Infusion into the Veins of the Scalp in Infants

O. SNELLMAN

Helsinki, Finland

To be published later in the *Acta Paediatrica*.

## 56. Intravenous Administration of Fat

OLLI KAUSTE  
Helsinki, Finland

The author has prepared a fat emulsion suitable for intravenous use. The composition of the emulsion is: olive oil 20%, glucose 5%, egglecithin 3% in distilled water.

The emulsion has a droplet size of less than  $2\ \mu$ , and a caloric content of 2,050 Cal./litre. Elevation of temperature,  $1^{\circ}\text{C}$  or more, during or after infusion, was observed in 5%, and other side effects in 3% of cases.

Intravenous therapy with this emulsion was given in about 50 cases with whom feeding by mouth was impossible or unsuccessful. The children were daily given 2–6 g fat pro kg body weight. In the exhibition, the author demonstrated one of the treated children who gained weight well on this regime.

Children of different ages and with metabolic disturbances of different types were given intravenous fat injections (0.5 g fat per kg body weight). The duration of the hyperlipemia was studied. In young infants and prematures the lipaemia lasted for a longer time than in older children. An extraordinary longlasting hyperlipemia was observed in the nephrotic syndrome. On the contrary, there was observed a very short lipemia in children suffering from hepatitis and cirrhosis of the liver.

## Scientific Films

*The following scientific films were shown at the Congress:*

- 1) Heart Cathetrisation and Angio-Cardiography with an Image Amplifier.  
HIRVONEN, L., GRIBBE, P. O., LIND, J., PELTONEN, T. and WEGELIUS, G.,  
Stockholm, Sweden and Turku, Finland.
- 2) The Abdominal Reflexes and their Clinical Significance in the Infant.  
HARLEM, O. K.  
Bergen, Norway.
- 3) A Case of Hypoglycaemia Treated by Resection of the Pancreas.  
GRIPENBERG, L. and SNELLMAN, O.  
Helsinki, Finland.
- 4) Physiological Studies in Connection with Prolonged Perfusions of Foeta  
from Legal Abortions.  
NYBERG and WESTIN.  
Stockholm, Sweden.

APPENDIX

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## **Rules of the Nordic Pediatric Association**

### *Section 1*

The object of the Association is to promote scientific and practical interest in pediatrics in the Nordic countries, in particular by holding congresses in the respective countries in turn. At these congresses only Members of the Association have the right to vote.

### *Section 2*

Every physician interested in pediatrics may, if proposed by the Council of the country concerned, be admitted to membership of the Association provided that he is a member of the pediatric branch or society of his country. Notice of application for and resignation from membership shall be made in writing to the secretary of the country in question.

The membership fee is fixed at the congress by the Members of the Association on the motion of the Board.

Honorary Members of the Association and of the Board may be appointed at congresses on the motion of the Board. Honorary Members and Members over sixtyfive years of age pay no membership fee.

### *Section 3*

The Board consists of four Members from each country, elected at a congress or other meeting of the Association. A Secretary is elected by each country from among the Board Members. The Secretary in the country where the congress is held will function as the Secretary-General and represent the Association until the following congress. He will edit and publish the transactions of the congress.

The Secretaries maintain a list of the Members of their countries, collect the membership fees and keep accounts. The financial year ends on March 31. The accounts for each individual country must be inspected and approved yearly by its Auditors.

During a congress year the Secretaries shall be responsible for sending to the Secretary-General within May at the latest a summary of the accounts for the years elapsed since the last congress. The Secretary-General shall draw up a joint statement of accounts for all the countries and this shall be inspected and signed by all the Auditors.

#### *Section 4*

Preparations for congresses shall be the responsibility of the Board and specifically of the Board Members in whose country the congress is to be held. A President will be responsible for the conduct of the transactions and will lay down the agenda; he will be assisted by the Vice-Presidents elected from the other countries represented at the congress.

The fee for each individual congress shall be fixed by the Council in the country in which the congress is held.

Physicians who are not members of the Association may also attend congresses against payment of a fee fixed by the Board.

#### *Section 5*

On the motion of the Board, the time and place of the next congress will be fixed at the congress, and the Secretary-General of the preceding congress or his deputy shall submit the accounts of the Association, duly audited, for approval.

The congress shall elect a President for the next congress.

In addition, an Auditor shall be elected from each country.

The agenda must be fixed and Members notified accordingly at least four months before the next congress.

#### *Section 6*

Proposed amendments to these rules must be put to the Board not later than one month before the congress of the Association meeting at which the proposals are to be discussed. Such amendments require two-thirds of the votes of the Members present.

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7. Valtquist, Bo Cson, Das Serum-eisen. Eine pädiatrisch-klinische und experimentelle Studie. Sw. kr. 10.—
8. Witon, Åke, Gewebsbiologische Studien über die Pathogenese der Addison-Biermer'schen Anämie. Sw. kr. 5.—
9. Hellitz, Gillis, Studien über die sog. initialen Fieberkrämpfe bei Kindern. Sw. kr. 5.—
10. Whilén, Olof S., Über die Methodik bei der Ausführung der Tuberkulinreaktionen sowie über die Verbreitung der Tuberkulinallergie bei Schulkindern im nördlichen Schweden (Västernorrland). Eine statistische Analyse. Sw. kr. 5.—
11. Brandberg, Olof, Studien über das klinische Bild der Leukosen und der sog. leukämoiden Reaktionen im Kindesalter. Sw. kr. 5.—
12. Eägren, Gunnar, Prognose und Erblichkeitsmomente bei Eczema infantum. Sw. kr. 5.—
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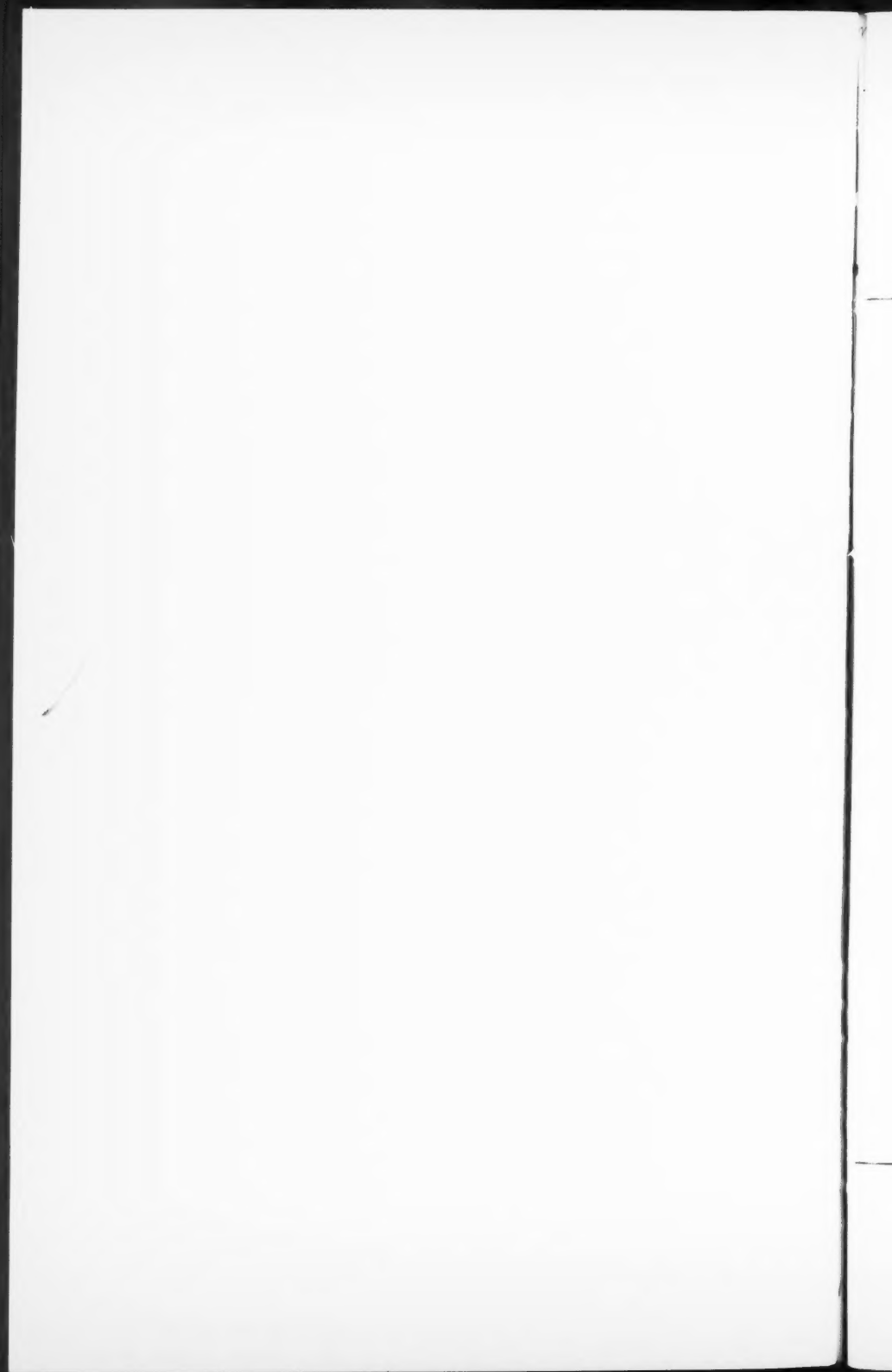
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**OBSERVATIONS ON ABO  
INCOMPATIBILITY BETWEEN  
MOTHER AND INFANT**

*By*

**G. C. ROBINSON, H. G. DUNN  
AND L. C. WONG**

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UNIVERSITY OF MICHIGAN

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From the Department of Pediatrics, Faculty of Medicine, University of British Columbia

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Observations on ABO Incompatibility between  
Mother and Infant

*by*

GEOFFREY C. ROBINSON, M.D., F.R.C.P.(C)\*

and HENRY G. DUNN, M.B., M.R.C.P., D.C.H.\*

*with the Collaboration of*

L.C. WONG, M.D.\*\*

*and with the Technical Assistance of*

J.M. McKENTY, R.T., and M. WILLMS, M.A.

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\* Assistant Professor (Drs. Robinson and Dunn)

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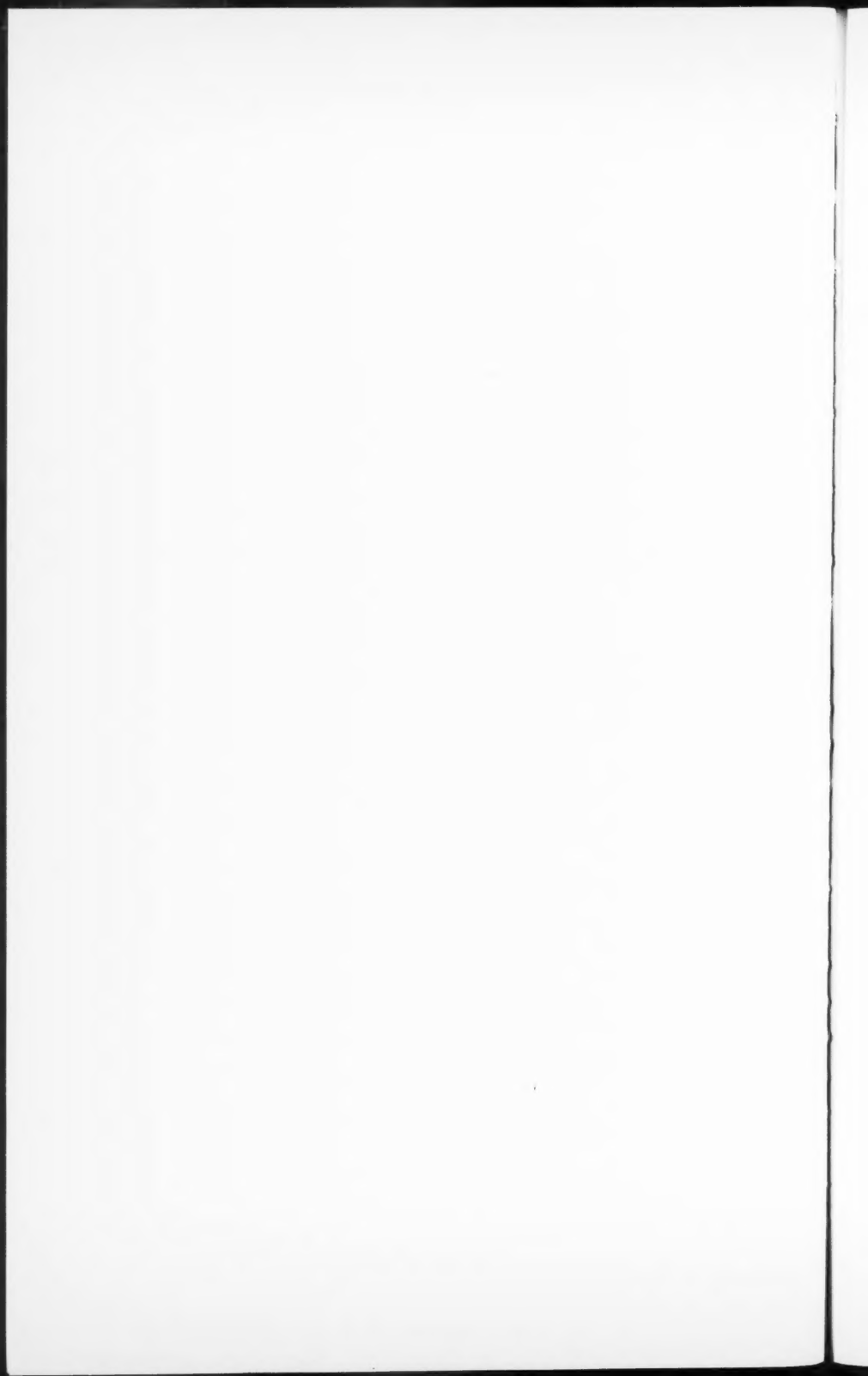
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Introduction

*and*

PART I

Patterns of Maternal ABO Antibodies in  
Unselected Pregnancies of Group O Women

*by*

GEOFFREY C. ROBINSON, M.D., F.R.C.P. (C)

HENRY G. DUNN, M.B., M.R.C.P., D.C.H.

*with the Technical Assistance of*

J. M. McKENTY, R.T.



## Introduction

One in every four to five pregnancies is "heterospecific" according to the present usage of that term. This means that the infant belongs to a different ABO blood group than the mother and has an agglutinin in his red cells which she does not possess, and she will naturally have the corresponding opposing isoagglutinin in her serum. The following list shows the six possible blood group relationships in heterospecific pregnancies.

<i>Maternal Blood Group</i>	<i>Infant's Blood Group</i>	<i>Maternal Agglutinin</i>
O	A or B	Anti-A, Anti-B
A	B or AB	Anti-B
B	A or AB	Anti-A

A priori, one might expect that hemolytic disease of the newborn is liable to occur in all such pregnancies owing to the effects of the opposing maternal agglutinin on her offspring. Clearly, the infant is usually shielded against harmful effects of these antibodies by a variety of factors (1), (2). However, the protective mechanisms are now known to be frequently incomplete.

Many investigators have studied this blood group relationship and its possible harmful effects on the infant, and the following main conclusions have emerged:

1. In heterospecific pregnancy, the level of the opposing naturally-occurring maternal agglutinin which is active in saline is liable to rise under the stimulus of the fetal antigen. This rise is quite small during pregnancy and is usually most marked after delivery; according to Boorman and her associates (3) it reaches its peak most

frequently between the 10th and 20th days postpartum.

2. A variety of "immune" antibodies have been described within the ABO system. These may be demonstrated by a shift in the thermal optimum of agglutination in saline from normal (4°C) to 37°C, or by the presence of "incomplete" agglutinins, hemolysins, opsonins, or of sensitizing antibodies which will cause agglutination of A or B cells in the presence of anti-human-globulin serum.

The shift in thermal optimum (4) (5) is a noteworthy but inconstant phenomenon.

Immune or "incomplete" agglutinins (Wiener's "glutinins") act only in colloid media, such as serum (6), acacia (7), or albumin which probably take part in the reaction.<sup>1</sup> They are difficult to demonstrate in the presence of naturally-occurring "saline-agglutinins" unless they are present to a higher titre; they can, however, be shown up if the saline-agglutinins are first neutralized by the addition of A or B group specific substance (8). Similarly, the sensitizing antibody may be demonstrated if the serum gives a positive indirect antiglobulin test with homologous cells, either to a higher titre than that of agglutination in saline or else after prior removal of saline-agglutinins by absorption with group specific substance. Hemolysins are demonstrated more easily and often very usefully (9). They have long been known

<sup>1</sup> In these articles the terms "saline-agglutinin" and "incomplete agglutinin" will be used for agglutinin acting in saline and only in a colloid medium, respectively.

to increase in titre postnatally in the maternal blood as a result of heterospecific pregnancy, somewhat like saline-agglutinins (10). Oponins (11) have not yet been used extensively for diagnostic purposes.

These "immune" antibodies are not present universally like saline-agglutinins, but they may all be found in normal persons who have not been sensitized by pregnancy or transfusion. Thus small amounts of anti-A incomplete agglutinins were found in 12% (12) and 22% (13) of random group O blood donors, and anti-A hemolysins have been still more widely encountered (14) (15).

3. At birth, the infant has not yet produced any anti-A or anti-B agglutinins of his own. Compatible maternal antibodies, including saline-agglutinins, cross the placenta readily and can be demonstrated in the infant's serum. In heterospecific pregnancy, incompatible saline-agglutinins can only rarely be found in the baby's serum and are probably largely removed or neutralized in the placenta (16). Incompatible incomplete (or "univalent") agglutinins reach the infant more often (17). Incompatible sensitizing antibodies also pass through the placenta frequently, thus causing the infant's serum to give a positive indirect anti-globulin test against adult red cells of his own group.

4. The great majority of infants born of heterospecific pregnancy appear clinically to be perfectly well. However, if one investigates cases of benign neonatal jaundice commencing within 24 hours of birth and unaccompanied by any blood change apart from occasional mild anemia, one finds that a very high proportion are

associated with maternal-fetal ABO incompatibility. In 1944 Halbrecht (18) reported 60 instances of this condition which he termed "*icterus neonatorum praecox*", and in 95% of these the pregnancy had been heterospecific. His work was confirmed by others (19) (20) (7), although the incidence of heterospecific pregnancy in subsequent similar series of patients was not quite as high.

5. A small minority of infants born of heterospecific pregnancy develop the full-blown picture of hemolytic disease of the newborn due to ABO iso-immunization. Many such cases have now been recorded, and the commonest symptomatology is evidently "*icterus gravis*" which may lead on to kernicterus. "*Anemia neonatorum*" is somewhat less frequent, and "*hydrops fetalis*" has only rarely been recorded (21) (22) (23) (24) (15). In nearly all these cases of hemolytic disease the mother belongs to Group O and the infant to A or B (25) (26) (27) (15). The disease differs somewhat from that due to Rh incompatibility. Thus in  $\frac{1}{3}$  to  $\frac{1}{2}$  of the ABO cases the mother is a primigravida; also the infant usually has hematological changes in the form of microspherocytosis and increased red cell fragility (23) (28).

6. It is now thought that "*icterus praecox*" represents only a mild form of hemolytic disease due to ABO iso-immunization (29). This has broadened the concept of the latter condition and implies that the disease is usually mild and is quite common, in fact more common than that due to Rh incompatibility (30) (26).

As one of us has pointed out previously (31), there are two main difficulties in estimating the true incidence of hemolytic disease caused by ABO antibodies. The

first is that of definition, for many cases are so mild as to shade off imperceptibly into early "physiological" icterus, with onset of jaundice after the first day. The second is that of serological diagnosis, for this may be suggestive but is rarely conclusive.

7. In regard to serological evidence, the presence of a greatly raised maternal anti-A or anti-B saline-agglutinin titre and/or the demonstration of maternal "immune" anti-A or anti-B antibodies are suggestive of the diagnosis of ABO hemolytic disease in the presence of a characteristic clinical and hematological picture in the child, when other blood group incompatibilities have been excluded. However, such changes may be present in a mother, and may even arise owing to immunization by the fetus, without necessarily causing any harm to the infant (32). So for diagnostic proof the presence, and preferably the action, of the offending antibody *in the infant* must be demonstrated. The finding of incompatible maternal incomplete agglutinin ("glutinin") or sensitizing antibody in the baby's serum supports the diagnosis, but evidence of attachment of antibodies to the baby's red cells is still better proof. Unfortunately, the direct antiglobulin test with commercial antiglobulin serum is usually negative or only weakly positive in infants affected by ABO hemolytic disease, so that this diagnostic tool has often been of little assistance. There remains the most convincing evidence of all, a reduced survival time of red cells of his own group transfused into the infant as compared to that of group O cells (33) (34) (35) (36), but this test is rarely practicable in routine work.

The serological methods of diagnosis in ABO hemolytic disease may thus be tabulated as follows:

I. Demonstration of ABO incompatibility between mother and child with exclusion of any maternal immunization in other blood group systems.

II. Demonstration of anti-A or anti-B saline-agglutinin in high titre in the maternal serum.

III. Demonstration of "immune" anti-A or anti-B antibodies in the maternal serum —

1. Saline-agglutinins, exhibiting optimal agglutination at 37°C.

2. Incomplete agglutinins, as shown by:

(1) agglutination at a greater dilution in colloid media than in saline,

(2) persistence of agglutination in colloid media, after neutralization of saline-agglutinins by group specific A or B substance.

3. Sensitizing antibodies, as shown by positive indirect antiglobulin test

(1) at higher titre than agglutination in saline, or

(2) after neutralization of saline-agglutinins by group specific A or B substance.

4. Hemolysins, active at 37°C.

5. Opsonins causing erythrophagocytosis.

IV. Demonstration of incompatible anti-A or anti-B antibodies in the infant.

1. Presence of incompatible antibodies in the serum.

(1) saline-agglutinins (rarely).

(2) incomplete agglutinins.

- (3) sensitizing antibodies,
- (4) hemolysins.
2. Positive direct antiglobulin test.
3. Reduced survival time of red cells of his own group transfused into the infant.

### Objects of Present Study

In order to elucidate the incidence and natural history of hemolytic disease due to ABO iso-immunization, we decided to perform a prospective study of a number of pregnant Group O women and their offspring. In this way it was hoped to compare the serological findings in regard to different forms of ABO antibodies in homospecific and heterospecific pregnancies, and in primigravidae and multi-gravidae, from early pregnancy to the puerperium. Further, it was planned to relate the maternal data to the clinical, hematological and serological status of the infants. The latter was to be compared with the findings in a separate group of babies presenting with frank hemolytic disease due to ABO iso-immunization.

### Methods<sup>1</sup>

For various reasons it was decided to omit the study of shifts in the thermal optimum of agglutination (III.1.) in the above list), of the maternal indirect antiglobulin test *without* neutralization (III.3. (1)), of opsonins (III.5) and of erythrocyte survival in the infants (IV.3). In view of difficulties in maintaining fresh supplies of

AB serum it was also decided to use 20 % bovine albumin<sup>2</sup> as a colloid medium in testing for immune agglutinins, and this proved quite adequate. The following methods were adopted:

*ABO Group and Rh Type.* The ABO groups of both mother and infant were determined by testing their cells against Anti-A and Anti-B sera, and their sera against known group A<sub>1</sub> and B cells. After the tubes had been kept at room temperature (20°C) for one hour, readings were made macroscopically.

The Rh types of mother and infant were determined by using saline-active antisera; in some cases anti-D, anti-C, anti-E and anti-c were used, but in the majority of instances only the D type was determined. The tubes were incubated at 37°C for 1½ hours before being read macroscopically.

In all instances 3 % suspensions of appropriate cells in saline were used and the tests were made in 6 × 50 mm tubes.

*Investigation of Maternal Sera for Irregular Antibodies.* All maternal sera were first tested against a pool of trypsinized group O cells from two or three persons with the probable Rh-genotypes CDe/cde and cDE/cde, and with as many other antigens known to be a potential cause of hemolytic disease of the newborn, as possible. Sera giving agglutination by this method were then investigated to determine the specificity and the titre of the antibody by the method next to be described, with the exception that in this instance the temperature of incubation was 37°C for both saline and albumin titrations.

*Method for Determining the Titre of Anti-A and Anti-B Agglutinins.* Doubling

<sup>1</sup> We are indebted to Dr. B. P. L. Moore, Director, National Laboratories, Canadian Red Cross Blood Transfusion Service, Toronto, Ontario, for working out the details of these techniques.

<sup>2</sup> Supplied by the armour laboratories.

dilutions of the serum were made from  $\frac{1}{5}$  up to  $\frac{1}{10,240}$  using a one drop technique in  $6 \times 50$  mm tubes. To each tube of one titration series in which saline had been used as the serum diluent, one drop of a 2% suspension of cells in saline was added and the tubes were kept for two hours at room temperature ( $20^{\circ}\text{C}$ ) before the reactions were read microscopically. In the other titration series, 30% bovine albumin was used as diluent, and the cells in 2% concentration were also suspended in this medium; the period of incubation was two hours at  $37^{\circ}\text{C}$ . The method of reporting the results is that described by Race and Sanger (37).

*Detection of Hemolysins.* Doubling dilutions of the serum were made from  $\frac{1}{2}$  to  $\frac{1}{16}$  using a two drop technique in  $10 \times 75$  mm tubes. Two drops of complement (reconstituted guinea-pig serum)<sup>1</sup> were added and one drop of a 20% suspension of washed group A<sub>1</sub> or B cells in saline. After 2 hours' incubation at  $37^{\circ}\text{C}$  the reactions were read, the end-point being a trace of pink throughout the supernatant. Each batch of complement was standardized with complement of known activity.

*Direct Antiglobulin Test.* (a) Routine. Two drops of antiglobulin serum were added to two drops of a twice washed 2% cell suspension in saline in a  $6 \times 50$  mm tube. The mixture was incubated for  $\frac{3}{4}$  hour at  $37^{\circ}\text{C}$  before being read macroscopically.

(b) Titration method using native serum. Dilutions of native (absorbed) rabbit serum<sup>2</sup> were made as follows: 1:10, 1:50,

1:100, 1:200 and 1:500, using a one drop technique. One drop of washed cells in 2% suspension was added to each tube. The mixture was incubated for  $\frac{3}{4}$  hour at  $37^{\circ}\text{C}$  and the reactions were read macroscopically.

To control both these methods, normal unsensitized red cells and red cells previously weakly sensitized with an incomplete anti-D serum were used with the same batch of antiglobulin serum.

*Indirect Antiglobulin Test.* Two drops of the serum to be tested were added to one drop of a  $\frac{1}{4}$ % suspension of washed cells in saline in a  $6 \times 50$  mm tube. Each test was set up in duplicate; one tube was incubated for one hour, the other for two hours at  $37^{\circ}\text{C}$ . After incubation the cells were washed three times in large volumes of saline, and treated as described above under Direct Antiglobulin Test (Routine).

*Detection of Incomplete Agglutinins.* Various proportions of A and B Group Specific Substances (G.S.S.)<sup>3</sup> and serum were used in order to obtain a mixture in which the saline-agglutinins were just neutralized.

In five  $12 \times 75$  mm tubes the following mixtures were prepared:

Parts of G.S.S.	1	2	3	4	7	9
Parts of Serum	1	1	1	1	1	1
Serum dilution	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{4}$	$\frac{1}{5}$	$\frac{1}{8}$	$\frac{1}{10}$

The tubes were shaken, left at  $20^{\circ}\text{C}$  for 5–10 minutes, then one drop of washed 2% suspension of appropriate cells in saline was added to one drop of each mixture and the tubes were incubated for one hour at  $37^{\circ}\text{C}$ . The reactions were read microscopically. If Group Specific Substances A and B did not neutralize the saline-agglutinins, Group Specific Polysaccha-

<sup>1</sup> Supplied by the Provincial Public Health Laboratories, British Columbia.

<sup>2</sup> Supplied by the Ortho Pharmaceutical Corp., Raritan, New Jersey.

<sup>3</sup> Supplied by Sharp and Dohme.

TABLE 1. *The incidence of the various possible combinations of "immune" anti-A antibodies in 63 female and 109 male group O blood donors.*

Anti-A hemolysin	After Neutralization		Female cases    %		Male cases    %		Total cases    %	
	Indirect AHG test	Incomplete agglutinin						
-	-	-	16	25	37	34	53	31
-	-	+	1	2	1	1	2	1
-	+	-	2	3	2	2	4	2
+	-	-	3	5	1	1	4	2
+	-	+	1	2	0	0	1	1
+	+	-	3	5	2	2	5	3
-	+	+	14	22	26	24	40	23
+	+	+	23	36	40	37	63	37
Total			63		109		172	

ride<sup>1</sup> A or B was tried diluted  $\frac{1}{20}$ , or, failing that,  $\frac{1}{10}$  until complete neutralization was obtained. The mixture showing "weak" or "negative" reactions was then titrated against appropriate A<sub>1</sub> or B cells in 20% bovine albumin, using a one drop technique in 6 × 50 mm tubes, as described above. An indirect antiglobulin test was performed on the first tube of the neutralized saline titration series showing no agglutination.

*Hemoglobin concentration* was determined as oxyhemoglobin using 0.007 N. NH<sub>4</sub>OH as diluent and a Klett-Summerson photoelectric colorimeter.

*Total Serum Bilirubin.*<sup>2</sup> The method of Malloy and Evelyn (38) was modified by adding 0.5 ml fresh Diazo Reagent (or Diazo Blank) to 1.5 ml of an aqueous dilution of serum (1 in 30) and then adding 2.0 ml methanol. After standing for 30 minutes the difference between the optical

densities of test and blank was measured at 550 mμ and the total serum bilirubin concentration calculated.

*Osmotic Fragility.* The method of Parpart *et al.* (39) was used. Readings were made with a Klett-Summerson photoelectric colorimeter.

*Examination of Blood Film.* Wright's stain was used on films made from fresh oxalated venous blood or from blood obtained by heelprick. Spherocytes were reported as number per oil immersion fields of 100 red cells (1+ = 1 to 2, 2+ = 2 to 3, 3+ = 3 to 4, 4+ = 4 or more).

### Preliminary Study

To determine the serological status of the local population and to gain experience with these methods, a number of random group O blood donors were tested initially. This study will not be reported in detail here, but the following points deserve mention:

(1) Among 69 female donors anti-A hemolysin in small amounts was found in

<sup>1</sup> Supplied by Sharp and Dohme.

<sup>2</sup> The authors are indebted to Dr. J. Eden, Chemical Pathologist, The Vancouver General Hospital, for this modification.

33 (48%) while anti-B hemolysin was detected in only 10 (14%). Of the 10 women with anti-B hemolysin, 7 also had anti-A hemolysin.

Similarly, among 112 male donors anti-A hemolysin in small amounts was found in 47 (42%), whereas anti-B hemolysin was detected in only 3 (3%), all of whom also had anti-A hemolysins.

This higher incidence of anti-A as compared to anti-B hemolysins in group O persons has been noted previously (13) (14).

(2) 63 female and 109 male group O donor sera were examined for anti-A hemolysin and both for incomplete anti-A agglutinins and by the indirect antiglobulin test against  $A_1$  cells after neutralization of saline-agglutinins. The results are listed in Table 1.

Thus there was a fair correlation between the incidence of the different forms of "immune" antibody, and 37% of all donors showed all three forms in their serum. Only 25% of the women and 34% of the men were completely "nonimmune" by these tests. Among the rest, the indirect antiglobulin and incomplete agglutinin tests were more often positive than the hemolysin, and in this regard the donors differed from the pregnant women examined later.

### Clinical Material

All women attending the Prenatal Clinic at The Vancouver General Hospital had ABO grouping and Rh-typing performed at their first visit. About half of these women were unmarried. All Rh-negative women with Rh antibodies and those of group A, B or AB, regardless of Rh type, were excluded from the study. In

this way a series of 154 group O women was collected. Further blood specimens were taken during subsequent clinic visits in the prenatal period, in hospital during the first week after delivery and at the subsequent clinic visit postnatally, usually about six weeks postpartum. Cord bloods were obtained from all infants. Each baby was examined by the same pediatrician daily until discharge.

Of the 154 women, 100 proved to have homospecific and 54 to have heterospecific pregnancies. In each group there was one pair of twins, so that 101 infants resulted from homospecific and 55 from heterospecific pregnancies. There were no stillbirths or neonatal deaths.

The heterospecific cases were studied fully, while in the homospecific group only 49 pregnancies were investigated in regard to the behavior of anti-A and 20 in regard to anti-B antibodies. To detect maternal agglutinin, titrations were performed in saline at room temperature and in albumin at 37°C. Maternal sera were also tested for hemolysins, and following complete neutralization with A and B substance, indirect antiglobulin tests were undertaken and agglutinin titration in albumin repeated. Cord bloods were examined and blood grouping, Rh-typing, hemoglobin determination, reticulocyte and nucleated red cell count, blood film studies and serum bilirubin determination were performed. Serological studies on the cord blood included agglutinin titrations in saline and albumin media, indirect antiglobulin tests of the infant's serum against adult group  $A_1$  or B cells, and direct antiglobulin tests. The direct tests were done using both commercial antiglobulin serum and various dilutions of native

antiglobulin serum as described in the section on Methods. Further hematological, biochemical and serological studies on the infants were undertaken as indicated by the initial serological findings and the clinical progress.

Finally, the postnatal maternal serological data and the findings in the infants were compared to those in 37 cases of proven hemolytic disease of the newborn due to ABO iso-immunization.

## PART I

# Patterns of Maternal ABO Antibodies in Unselected Pregnancies of Group O Women

### Results

#### 1. Homospecific Pregnancies

100 homospecific (O-O) pregnancies were studied, and these resulted in 101 group O infants because of one twin birth. Maternal and infant cord serological studies of the anti-A antibodies were performed in 49 of these cases, and of the anti-B antibodies in an additional 20 cases.

(1) *Saline-Agglutinins.* The results of prenatal and postnatal serological studies in this group are set out in Tables 2 and 3. The range of anti-A saline-agglutinin titres was broad prenatally as well as in the early<sup>1</sup> and late postnatal periods. The median of the saline titres suggests a slight postnatal rise in some cases, and this is

<sup>1</sup> In these articles the early postnatal period refers to the first week after delivery. The late postnatal period extends from start of second week to the end of the second month.

borne out by an analysis of the titre changes, relative to the original level, as illustrated in Tables 4 and 5. No marked change in antibody titres in saline is evident on multiple prenatal testing, but a definite upward trend of titres appeared to occur after delivery although the numbers are too small to attain significance on the  $X^2$  test. Unfortunately only 3 out of the 10 women who had a rise of at least two tubes in their anti-A saline titre after delivery had more than one postnatal check of the titre; in all 3 of them the saline anti-A level rose to its highest level after the first week of the puerperium, as described by Boorman and her colleagues (6).

The titres and fluctuations in primigravidae did not differ essentially from those of multigravidae. The levels are shown in Fig. 1, and their changes are indicated in Table 4.

TABLE 2. *Prenatal and postnatal maternal anti-A agglutinin titres in saline at room temperature in homospecific (O-O) and heterospecific (O-A) pregnancies.*

	Homospecific (O-O)			Heterospecific (O-A)		
	No. of patients	Range	Median	No. of patients	Range	Median
<i>Prenatal</i>						
Saline	49	20-10,240	320	39	40-10,240	320
<i>Early postnatal</i>						
Saline	41	40-81,920	640	31	40-81,920	640
<i>Late postnatal</i>						
Saline	20	160-20,480	640	16	640-20,480	1280

TABLE 3. *Prenatal and postnatal maternal anti-B agglutinin titres in saline at room temperature in homospecific (O-O) and heterospecific (O-B) pregnancies.*

	Homospecific (O-O)			Heterospecific (O-B)		
	No. of patients	Range	Median	No. of patients	Range	Median
<i>Prenatal</i>						
Saline	20	20-5120	320	14	20-1280	160
<i>Early postnatal</i>						
Saline	18	20-10,240	320	12	40-10,240	320
<i>Late postnatal</i>						
Saline	10	20-2560	320	4	320-40,960	

The maternal anti-B titres in saline are shown in Table 3 and appeared somewhat lower than those of anti-A. The levels are shown in Fig. 2. The number of women who had their anti-B titres checked repeatedly during homospecific pregnancies is small, but as shown in Table 5 there is again a trend towards a rise.

It will be seen from Table 4 that a total of 10 out of 49 mothers (20.0%) had a significant (two tube) rise of anti-A saline-agglutinin titres from the antenatal to the postnatal period, while only one had a significant fall.

(2) *Agglutinins in Albumin.* Boorman, Dodd and Morgan (6) first demonstrated

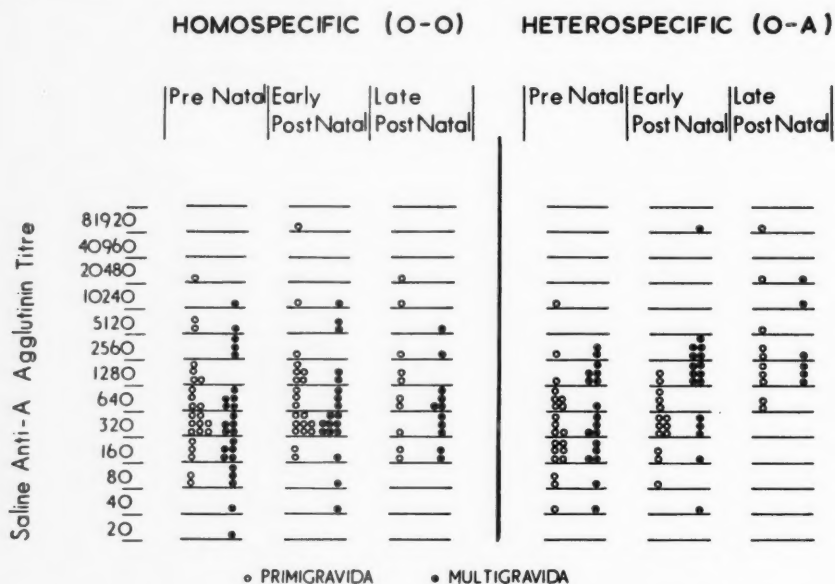


Fig. 1. Saline anti-A agglutinin titres in homospecific pregnancy compared with those of heterospecific (O-A) pregnancy during prenatal and postnatal periods.

TABLE 4. (a) Multiple prenatal testing and (b) comparison of highest prenatal and postnatal titres in homospecific (O-O) and heterospecific (O-A) pregnancies.

	Total number of patients tested	Titres unchanged on repeat testing	Rise		Fall	
			1 tube	2 tube	1 tube	2 tube
(a) Multiple prenatal anti-A titres						
<i>Homospecific (O-O)</i>						
In saline at room temp. -	24 (10)*	13 (6)	5 (3)	1 (0)	5 (1)	0 (0)
<i>Heterospecific (O-A)</i>						
In saline at room temp. -	16 (10)	5 (4)	5 (3)	4 (1)	2 (2)	0 (0)
(b) Comparison of highest prenatal and postnatal anti-A titres						
<i>Homospecific (O-O)</i>						
In saline at room temp. -	49 (22)	26 (9)	9 (5)	10 (6)	3 (1)	1 (1)
<i>Heterospecific (O-A)</i>						
In saline at room temp. -	34 (18)	10 (4)	5 (2)	14 (10)	4 (1)	1 (1)

\* Figures in parentheses refer to primigravidae

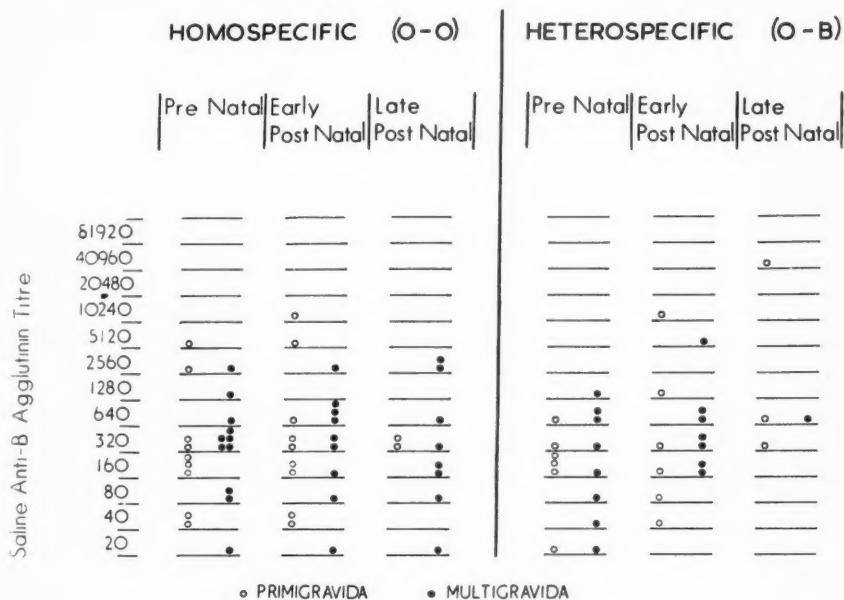


Fig. 2. Saline anti-B agglutinin titres in homospecific pregnancy compared with those of heterospecific (O-B) pregnancy during prenatal and postnatal periods.

TABLE 5. (a) Multiple prenatal testing and (b) comparison of highest prenatal and postnatal titres in homospecific (O-O) and heterospecific (O-B) pregnancies.

	Total number of patients tested	Titres unchanged on repeat testing	Rise		Fall	
			1 tube	2 tube	1 tube	2 tube
<i>(a) Multiple prenatal anti-B titres</i>						
<i>Homospecific (O-O)</i>						
In saline at room temp. -	12	4	4	2	2	0
<i>Heterospecific (O-B)</i>						
In saline at room temp. -	5	2	2	0	1	0
<i>(b) Comparison of highest prenatal and postnatal anti-B titres</i>						
<i>Homospecific (O-O)</i>						
In saline at room temp. -	20	11	6	0	3	0
<i>Heterospecific (O-B)</i>						
In saline at room temp. -	13	2	4	6	1	0

the presence of immune ABO agglutinins in 1945 by the fact that the addition of human serum enhanced agglutination. As previously mentioned, we used albumin as diluent instead of serum to demonstrate such a qualitative difference in agglutination. It is realized that the actual titres in albumin at 37°C are of little qualitative significance in regard to the presence of incomplete agglutinins, unless the titre exceeds that of saline-agglutinins by at least two dilutions, and even if it does so it may represent a shift in the thermal optimum for saline-agglutinins rather than the presence of incomplete antibodies requiring the presence of colloid media for their reaction. Nevertheless, it appeared to us that this test might act as a rough screening test in detecting incomplete agglutinins.

Among 36 women tested in the prenatal period there were six in whom the excess of the anti-A titre in albumin at 37° over

the saline anti-A titre at room temperature was at least two dilutions (Case Nos. 20, 28, 29, 36, 38 and 40).<sup>1</sup> In three of these cases (Case Nos. 28, 36 and 38)<sup>1</sup> this difference persisted into the postpartum period. This suggested the presence of an immune anti-A, and additional evidence for this was available in each case by the presence of incomplete agglutinins or sensitizing antibody after neutralization of the saline-agglutinin. Another woman (Case No. 7)<sup>1</sup> developed a markedly increased titre in albumin after the first postpartum week, together with other evidence of immune anti-A as demonstrated after neutralization of saline-agglutinin. In one further woman (Case No. 35)<sup>1</sup> the albumin titre was not tested prenatally and a two tube difference was present six weeks after delivery. Thus a total of five mothers out of 39 tested postnatally exhibited this phenomenon. In all these five cases the

<sup>1</sup> See Part II, Table 13.

TABLE 6. *Comparison of prenatal and postnatal anti-A hemolysins in homospecific (O-O) and heterospecific (O-A) pregnancies. A rise or fall here means a change in titre of one tube or more.*

Homospecific pregnancy (O-O)	
Prenatal	Postnatal
Tests for anti-A hemolysin negative in 12 (3)*	5 (2) of these remained negative 7 (1) became positive
Tests for anti-A hemolysin positive in 32 (15)	These all remained positive. The titre was stationary in 20 (10), rose in 9 (3), fell in 3 (2).
Heterospecific pregnancy (O-A)	
Prenatal	Postnatal
Tests for anti-A hemolysin negative in 5 (3)	These all became positive
Tests for anti-A hemolysin positive in 16 (9)	These all remained positive. The titre was stationary in 8 (6), rose in 7 (2), fell in 1 (1)

\* Primigravidae in parentheses

post-neutralization studies also revealed at least one type of immune antibody (Case Nos. 7, 28, 35, 36 and 38)<sup>1</sup>.

The number of cases in whom anti-B titres in albumin was estimated is small. In the prenatal period the albumin titre was two tubes in excess of the saline titre in only one out of 20 tested (Case No. 3).<sup>2</sup> This difference was nullified in the postnatal period by a rise in the saline anti-B. No other cases of albumin anti-B excess developed after birth in 20 cases studied.

(3) *Hemolysins.* Maternal anti-A hemolysins were demonstrated in 32 and were absent in 12 out of 44 women in the prenatal period, compared to 30 positive and 6 negative out of 36 in the first postnatal week. Only 17 sera were

available for testing in the late postnatal period, and hemolysins were found to be present in 15 and absent in 2. The overall changes in 44 women whose blood was tested for the presence of anti-A hemolysins both before and some time during the first two months after delivery are shown in Table 6. There is a distinct trend for these anti-A hemolysins to appear if they are initially absent during pregnancy, and there is also a suggestion that their titre tends to increase after delivery if they are present at the outset.<sup>3</sup>

The range of hemolysin titre was 2-16 during each time period and was generally higher for anti-A than for anti-B. There is a suggestion in Table 7 that the anti-B hemolysin is not affected by pregnancy and delivery to the same extent as the anti-A variety.

The findings in regard to hemolysins in primigravidae and multigravidae did not appear to show any difference; this has

<sup>1</sup> See Part II, Table 13.

<sup>2</sup> See Part II, Table 14.

<sup>3</sup> For purposes of this comparative analysis one tube changes in titre are accepted as rise or fall.

TABLE 7. *Comparison of prenatal and postnatal anti-B hemolysins in homospecific (O-O) and heterospecific (O-B) pregnancies. A rise or fall here means a change in titre of one tube or more.*

Homospecific pregnancy (O-O)	
Prenatal	Postnatal
Tests for anti-B hemolysin negative in 5	3 of these remained negative 2 became positive
Tests for anti-B hemolysin positive in 19	2 became negative 17 remained positive. The titre was stationary in 12, rose in 1, fell in 4.
Heterospecific pregnancy (O-B)	
Prenatal	Postnatal
Tests for anti-B hemolysin negative in 4	3 of these remained negative* 1 became positive.
Tests for anti-B hemolysin positive in 1	This remained positive.

\* It is of interest that anti-A hemolysin became demonstrable in 2 of these and was present throughout in the third.

been previously emphasized by Jungwirth (40).

(4) *Indirect Antiglobulin Test and Incomplete Agglutinin after Neutralization.* Table 8 shows that in the prenatal period the sera of 15 women out of 48 were positive both for the indirect antiglobulin test and the presence of incomplete agglutinin in albumin against adult  $A_1$  cells after neutralization; in 5 cases the indirect antiglobulin test was positive, but incomplete agglutinin was not demonstrable; in 5 further cases the reverse was true; in 23 patients both tests were negative (though it is noteworthy that 6 sera contained saline-agglutinins which were hard to neutralize, even with 9 parts of concentrated group specific substance per 1 part serum). In all but 4 cases there was no change in regard to these reactions during and after delivery. One woman had neither incomplete agglutinin nor

sensitizing antibody after neutralization in the prenatal period and acquired both postnatally; another woman who had incomplete agglutinin with a negative indirect antiglobulin test after neutralization prenatally proved to have both tests positive postnatally; two other women, one of whom had a positive and the other a negative indirect antiglobulin test, appeared to lose the incomplete agglutinin postnatally which they had previously possessed, but in both cases increased amounts of group specific substance were required for neutralization.

Thus, it might appear that the "immune" anti-A, as demonstrated after neutralization, showed little change in consequence of pregnancy. It is, however, interesting to analyze the variations in the amount of group specific substance which were required for complete neutralization of the saline-agglutinin in these cases of

TABLE 8. Comparison of various possible combinations of two forms of immune anti-A antibodies after complete neutralization during prenatal and postnatal period in homo-specific (O-O) and heterospecific (O-A) pregnancies.

Indirect AHG test against A <sub>1</sub> cells	Test for incomplete anti-A agglutinin	Number of women in each group	
After complete neutralization of saline anti-A		Prenatal	Postnatal
<i>Homospecific pregnancy (O-O)</i>			
Negative	Negative	23	23
Positive	Negative	5	6
Negative	Positive	5	3
Positive	Positive	15	16
Total		48	48
<i>Heterospecific pregnancy (O-A)</i>			
Negative	Negative	14	12
Positive	Negative	3	4
Negative	Positive	3	3
Positive	Positive	15	16
Total		35	35

homospecific pregnancy. These amounts vary from two parts of dilute to nine parts of concentrated group A substance per one part of serum. Comparing the amounts required postnatally to those required before delivery we find that more group specific substance was required in 10 cases, the same amount was required in 33 cases, less was required in 5 cases.<sup>1</sup> This again suggests a slight tendency towards an increase in the amount of antibody postpartum, at least in regard to the saline-agglutinin. It seems likely that some of the "immune" antibodies also become neutralized by the addition of large amounts of group specific substance when complete neutralization of saline-agglutinin is attempted.

Table 9 shows the results of the above two tests against B cells after neutralization, and it is noted that no change in "immune" anti-B was detected in the

<sup>1</sup> One case not tested in prenatal period.

prenatal or postnatal studies. Comparing the amounts of group specific substance we find that five required more, 11 required the same amount and three required less in the postnatal period.

When the presence of hemolysins is analyzed, together with that of the sensitizing and incomplete agglutinating antibodies after neutralization (Table 10), it is evident that hemolysins were demonstrated more frequently in the sera of these women than in blood donors (see Table 1). On the other hand, sensitizing and incomplete agglutinating antibodies after neutralization were demonstrated in fewer cases, but possibly because of the larger amounts of group specific substance used for neutralization.

In summary, in homospecific pregnancy the level of saline anti-A and anti-B agglutinins showed a slight tendency to rise which was fairly definite after delivery. The titres of agglutinins in albumin at

TABLE 9. *Comparison of various possible combinations of two forms of immune anti-B antibodies after complete neutralization during prenatal and postnatal period in homo-specific (O-O) and heterospecific (O-B) pregnancies.*

Indirect AHG test against B cells	Test for incomplete anti-B agglutinin	Number of women in each group	
After complete neutralization of saline anti-B		Prenatal	Postnatal
<i>Homospecific pregnancy (O-O)</i>			
Negative	Negative	4	4
Positive	Negative	4	4
Negative	Positive	7	7
Positive	Positive	4	4
Total		19	19
<i>Heterospecific pregnancy (O-B)</i>			
Negative	Negative	1	3
Positive	Negative	1	1
Negative	Positive	4	5
Positive	Positive	7	4
Total		13	13

37° are more difficult to interpret; they were seldom distinctly higher than the saline titres at room temperature and thus did not indicate the presence of immune antibodies with certainty. A two tube excess of anti-A titre in albumin at 37°C over that in saline at room tem-

perature was only present in 6 of 36 cases prenatally and in 5 of 39 postnatally. Tests for hemolysins were positive in a remarkably large proportion of these pregnant women; thus anti-A hemolysins were found in 32 out of 44 women prenatally, and in 39 of the 44 postpartum.

TABLE 10. *The incidence of the various possible combinations of three forms of "immune" anti-A antibody in the same women in the pre- and postnatal periods in homospecific (O-O) and heterospecific (O-A) pregnancies.*

Maternal "immune" antibodies

Anti-A hemolysin	After neutralization		Homospecific pregnancy (O-O)		Heterospecific pregnancy (O-A)	
	Indirect AHG test	Incomplete agglutinin	Number of women in each group		Number of women in each group	
			Prenatal	Postnatal	Prenatal	Postnatal
-	-	-	7	4	4	0
-	-	+	1	1	0	0
-	+	-	1	1	1	0
+	-	-	15	19	6	10
+	-	+	4	2	1	1
+	+	-	4	4	3	3
-	+	+	3	0	1	0
+	+	+	8	12	7	9
Total			43	43	23	23

TABLE 11. *Marked postnatal rises in anti-A saline-agglutinins in 8 heterospecific (O-A) pregnancies with multiple testings. The horizontal lines in column 4 indicate the interval when delivery occurred. (P = primigravida, M = multigravida, NT = not tested.)*

Case No.	Parity	Birth date	Test dates	Saline agglutinin titre	Hemolysin		Parts to* neutralize	After neutralization	
					Anti-A	Anti-B		Indirect AHG test	Incomplete agglutinin
1	P	Oct. 23/54	Oct. 15/54	160	—	NT	3 dil.	—	Nil
			Oct. 27/54	320	4	NT	3 dil.	—	Nil
			Dec. 6/54	640	8	NT	3 dil.	—	Nil
5	P	Jan. 27/55	Nov. 19/54	320	—	NT	3 dil.	—	Nil
			Dec. 21/54	320	4	NT	3 dil.	—	Nil
			Jan. 31/55	640	4	NT	3 dil.	—	Nil
			Feb. 2/55	640	8	NT	3 dil.	—	Nil
			Mar. 7/55	1280	4	NT	9 dil.	—	Nil
6	P	Mar. 12/55	Jan. 24/55	40	—	NT	2 dil.	—	Nil
			Mar. 17/55	320	4	NT	2 dil.	—	Nil
			Apr. 22/55	5120	NT	NT	3 dil.	—	24
11	P	Dec. 10/54	Nov. 12/54	160	4	NT	3 dil.	+	32
			Dec. 16/54	160	4	NT	3 dil.	+	8
			Jan. 21/54	1280	4	NT	4 dil.	+	40
20	P	Jan. 9/56	Oct. 3/55	80	8	4	4 dil.	—	20
			Jan. 11/56	160	4	4	4 dil.	—	40
			Feb. 27/56	2048	16	4	11 conc.	—	Nil
21	M	Feb. 19/56	Feb. 11/56	160	8	4	3 dil.	—	Nil
			Feb. 19/56	320	8	4	5 conc.	—	Nil
			Mar. 9/56	20480	16	4	2 conc.	+	48
47	P	Jan. 15/56	Dec. 15/56	160	—	—	9 dil.	+	640
			Jan. 19/56	1280	16	8	3 conc.	+	32
			Feb. 27/56	4096	16	4	11 conc.	—	Nil
49	M	Feb. 7/56	Dec. 23/55	160	4	4	2 dil.	+	192
			Jan. 20/56	160	4	8	4 dil.	+	160
			Feb. 9/56	160	4	4	4 dil.	+	80
			Feb. 23/56	1280	32	16	11 conc.	+	48

\* Amounts of A or B substance required for complete neutralization of standard amount of serum. (dil. = group specific substance, conc. = group specific polysaccharide.)

Not only the proportion of positives, but also the titres tended to rise after delivery. The changes in anti-B hemolysins were less definite. The amounts of sensitizing and incomplete agglutinating antibodies in the serum after neutralization did not change significantly in homospecific pregnancy; the results, however, indicate a

slight tendency for increased amounts of group specific substance to be required for neutralization after delivery.

There appeared to be no significant difference between primigravidae and multigravidae in regard to the level or fluctuations of the various types of ABO antibodies in homospecific pregnancy.

TABLE 12. *Marked postnatal rises in anti-B saline-agglutinins in 3 heterospecific (O-I') pregnancies with multiple testings. The horizontal lines in column 4 indicate the interval when delivery occurred. (P = primigravida, M = multigravida, NT = not tested.)*

Case No.	Parity	Birth date	Test dates	Saline agglutinin titre	Hemolysin		Parts to* neutralize	After neutralization	
					Anti-A	Anti-B		Indirect AHG test	Incomplete agglutination
29	P	Apr. 9/55	Dec. 20/54	160	8	NT	4 dil.	—	40
			Feb. 14/55	160	4	NT	NT	NT	NT
			Apr. 4/55	320	NT	NT	18 conc.	—	80
			Apr. 14/55	1280	NT	NT	NT	NT	NT
			May 30/55	1280	NT	NT	32 conc.	—	1024
30	P	July 23/55	May 20/55	160	NT	NT	9 dil.	+	40
			July 27/55	10,240	NT	NT	9 conc.	+	160
			Sep. 2/55	40,960	16	16	59 conc.	—	60
36	M	Jan. 2/55	Nov. 5/54	20	4	—	NT	NT	NT
			Jan. 7/55	320	16	8	2 conc.	+	24
			Feb. 14/55	640	4	2	2 conc.	+	24
			July 29/55	160	—	—	14 conc.	—	32

\* Amounts of A or B substance required for complete neutralization of standard amount of serum. (dil. = group specific substance, conc. = group specific polysaccharide.)

## II. Heterospecific Pregnancies.

54 heterospecific pregnancies were studied; in 40 of these there was an O-A and in 14 an O-B relationship between mother and infant. 41 group A and 14 group B babies were born, as a twin birth occurred in one of the O-A pregnancies. Serological studies were performed in the same manner as in the homospecific group, and particular attention was paid to the maternal antibodies antagonistic to the infant's group.

(1) *Saline-Agglutinins.* The maternal anti-A titres in saline before and after delivery are shown on the right half of Table 2. The ranges in both prenatal and postnatal periods are broad, and the medians show a definite shift to higher titres. A detailed analysis of titre change is shown graphically in Fig. 1. It is evident that the rise is more marked than in

homospecific pregnancies, and that maximum titres are generally reached after the first postpartum week, as previously demonstrated by Boorman and her colleagues (6).

Multiple prenatal testings were performed in 16 women in O-A pregnancies who had a total of 39 prenatal titre estimations. Table 4 indicates a definite upward trend in these readings, even before delivery, both in primigravidae and in multigravidae. In four cases there was a rise amounting to a two tube difference, and it is of interest that two of the four infants born of these pregnancies had a positive direct antiglobulin test. One of these four women was a primigravida, and she had one of the latter two infants.

The highest maternal postnatal anti-A titre exceeded the maximal prenatal titre by two tubes or more in 14 out of 34 cases,

and this included 10 out of 18 primigravidae who were thus tested. In 8 of these marked rises at least two postnatal estimations of maternal anti-A are available, and these are listed in Table 11. The titres in primigravidae did not differ essentially from those in multigravidae as shown by Fig. 1.

The number of O-B heterospecific pregnancies in which estimations of anti-B were undertaken is shown in the right half of Table 3 and is too small for detailed evaluation. The maternal anti-B titres were lower than those of anti-A, as was also noted in the homospecific series. However, in 13 patients the maximal prenatal and postnatal anti-B titres are compared in Table 5, and it is interesting to find that no fewer than 6 of these showed a rise of at least two dilutions. Details of three of these cases, in whom multiple postnatal testings are available, are given in Table 12, and it is evident that the changes in anti-B resemble those of anti-A in O-A pregnancies. Again the titres in primigravidae did not differ essentially from those for multigravidae (Fig. 2).

A significant (two tube) rise in saline anti-A or anti-B titre from the prenatal to the postnatal period thus occurred in a total of 20 out of 47 mothers (42.5%) as listed in Tables 4 and 5. Of these 14 out of 37 (38%) were significant rises of anti-A in O-A cases.

(2) *Agglutinins in Albumin*. As previously mentioned the results of anti-A and anti-B titrations in albumin at 37°C are difficult to interpret. In general the range and median during the different time periods resembled that in saline.

<sup>1</sup> See Part II, Table 15.

The titre in albumin exceeded that in saline by at least two dilutions in 5 of 37 O-A (Case Nos. 25, 26, 27, 47 and 50)<sup>1</sup> and 3 of 14 O-B cases (Case Nos. 32, 39 and 54)<sup>1</sup> tested for both prenatally, thus suggesting the presence of an "immune" agglutinin in 8 of 51 mothers. In at least one of these (Case No. 27)<sup>1</sup> this state was shown to develop during the prenatal period. In 7 of the 8 there was other evidence of immune anti-A after neutralization of saline agglutinin. Postnatally one of these 8 mothers was not tested again (Case No. 54).<sup>1</sup> The remaining 7 retained this significant excess of albumin over saline titre during the puerperium, and an additional 3 cases (Nos. 6, 20 and 49)<sup>1</sup> acquired it in the first postnatal month, together with other evidence of immune anti-A after neutralization of saline-agglutinin. Hence 10 out of 48 women tested postnatally (8 of 36 O-A and 2 of 12 O-B cases) showed this phenomenon. Of the total of 11 women who exhibited the difference at some phase before or after birth, 2 had infants affected by hemolytic disease (Nos. 50 and 54).<sup>1</sup>

(3) *Hemolysins*. In the O-A relationship between mother and infant anti-A hemolysins were demonstrated in the maternal serum in 16 out of 21 cases tested prenatally (76%). This may be compared to similar findings in homospecific pregnancies when 32 out of 44 maternal sera (73%) showed anti-A hemolysins. In 3 of our heterospecific cases (Case Nos. 12, 27, 48)<sup>1</sup> who exhibited marked prenatal elevations of the agglutinin titre in saline or albumin, the level of hemolysin also rose before delivery. However, the number of cases with multiple prenatal testing is too small to indicate the incidence of this

phenomenon. On the other hand, there appears to be a very definite change postnatally, as shown in Table 6. The five mothers out of 21 with no anti-A hemolysins during pregnancy all exhibited positive tests postpartum, and of the 16 who had had hemolysins before delivery, 7 subsequently showed a rise in titre. Thus the trend towards positivity of hemolysins and towards an increase of titre is more marked than in homospecific pregnancy. This phenomenon appears to affect both primigravidae and multigravidae.

The changes in anti-B hemolysins were studied in fewer cases and appeared less impressive (Table 7). In O-B heterospecific pregnancy only one out of five women exhibited anti-B hemolysins in her serum before delivery. Three out of the four who had negative tests prenatally remained negative subsequently, but it is of interest that *anti-A* hemolysin was present throughout in one of these and became demonstrable postpartum in the other two.

Crawford and his colleagues (41) have suggested the use of fetal cells from cord blood rather than adult cells in testing for hemolysins. In our series of heterospecific pregnancies maternal hemolysins against fetal red cells were determined in 14 women. In 3 of these hemolysis was demonstrated against adult but not against fetal cells, and in 6 the titre was lower against fetal than against adult  $A_1$  cells, while the titres were equal in the remaining five. A high titre of hemolysins against fetal cells may certainly suggest the possibility of damage to the baby; in our small experience the maternal titre against fetal A cells in heterospecific pregnancies was 8 or more in 4 cases, and

in 2 of these the infant had a positive direct antiglobulin test (Case Nos. 48 and 50).<sup>1</sup>

(4) *Indirect Antiglobulin Test and Incomplete Agglutinin after Neutralization.* During pregnancy the sera of 15 out of 35 group O women who subsequently had group A infants, gave a positive indirect antiglobulin test against  $A_1$  cells and also agglutinated the cells in an albumin medium after neutralization (Table 8). In 3 women agglutination in albumin after neutralization also occurred, yet the indirect antiglobulin test was negative, while in 3 the reverse was true. In 14, both tests were negative. These proportions are not markedly different from those previously described in homospecific pregnancy, although the proportion exhibiting both types of immune antibody was slightly higher in the heterospecific series. As shown in Table 8, there was little change in these figures after delivery. Actually, one woman (Case No. 6)<sup>1</sup> was found to have incomplete agglutinins after neutralization during the puerperium, while she had not had them previously, and two others (Case Nos. 18 and 21)<sup>1</sup> acquired both incomplete agglutinins and a positive indirect antiglobulin test after neutralization. On the other hand one woman (Case No. 42)<sup>1</sup> no longer had a demonstrable incomplete agglutinin after neutralization postpartum, while this had previously been present, and another (Case No. 47)<sup>1</sup> appeared to lose both the incomplete agglutinin and the positive indirect antiglobulin test after neutralization. However, in these two cases considerably larger amounts of group specific

<sup>1</sup> See Part II, Table 15.

substance had to be added for neutralization than before.

Thus, the possible increase in immune antibodies after delivery may well be masked by their absorption and dilution with the larger amounts of group specific substance required for neutralization of saline-agglutinins. In cases of O-A pregnancy the amount of group specific substance required for complete neutralization after delivery was greater than in the prenatal period in 15 cases, equal in 17 cases and less in only 2 cases.

In O-B heterospecific pregnancy the findings were analogous (Table 9). The indirect antiglobulin test was negative postnatally in 3 cases in whom it was positive in the prenatal period, and in each instance a greatly increased amount of group specific substance was required for neutralization. Thus, the proportion of cases requiring an increased amount of group specific substance for neutralization after delivery is larger in the heterospecific (O-A and O-B) than in the homospecific (O-O) group.

A correlation of all three forms of immune anti-A, namely hemolysin, and both indirect antiglobulin test and incomplete agglutinin after neutralization, in women who have had all three forms examined both pre- and postnatally, is set out in Table 10. This shows that the commonest form of immune antibody in this series of women was the hemolysin, which was present in about  $\frac{3}{4}$  of women in both homospecific and heterospecific pregnancy prenatally and was found in 36 out of 43 mothers with homospecific pregnancy and in *all* of 23 mothers in heterospecific pregnancy after delivery. The indirect antiglobulin test and incomplete agglu-

tinin after neutralization were positive in fewer cases; thus the indirect antiglobulin test after neutralization was positive against A<sub>1</sub> cells prenatally in 16 of 43 women in homospecific and in 12 of 23 women with heterospecific pregnancy (O-A); the incomplete agglutinins after neutralization were demonstrable again in 16 out of 43 mothers prenatally in homospecific pregnancy and in 10 of 23 in heterospecific pregnancy (O-A). As previously mentioned there was little change in these figures after delivery, though often larger amounts of group specific substance had to be used for neutralization. All forms of immune anti-A were present simultaneously in 8 out of 43 homospecific and 7 out of 23 heterospecific pregnancies prenatally; after delivery they were encountered together in 12 out of 43 homospecific pregnancies and in 9 of 23 heterospecific (O-A) pregnancies. Complete absence of immune anti-A in all three forms was found prenatally in about one-sixth of these women; after delivery this non-immunity was encountered in 5 out of 43 homospecific and in none of 23 heterospecific pregnancies.

In summary, in heterospecific pregnancy the titre of saline-agglutinins at room temperature shows a distinct tendency to rise even during the prenatal period and this becomes quite marked after delivery in nearly half the cases. This appears to occur equally in primigravidae and multi-gravidae. We have fewer figures concerning the anti-B than the anti-A agglutinins, but the trend appears to be similar. The titres of agglutination in albumin at 37° are more difficult to interpret but again show the same trend. Occasionally the titre in albumin rose to markedly higher

levels than that in saline, particularly during the postnatal period, thus suggesting an immune response. As suggested by Boorman *et al.* (6) the titres in saline appeared to reach their highest level after the first postnatal week and the same is probably true of the titres in albumin at 37°. An immune response may also be indicated by the increase in anti-A hemolysins which appear to rise from an incidence of about 75% of maternal sera prenatally to 100% postnatally and, when present, tend to show an increase of titre. We have fewer data concerning anti-B hemolysins, but these do not show such impressive changes. The incidence of residual agglutination in albumin after neutralization of saline-agglutinin as well as the finding of a positive indirect antiglobulin test after neutralization, does not appear to increase markedly as the result of heterospecific pregnancy, but such an increase might well be masked when complete neutralization is attempted as in our series. Such complete neutralization often proves difficult, and it was interesting that increased amounts of group specific substance were frequently required for neutralization after delivery.

### Discussion

Dienst (42) first showed that the titre of anti-A and anti-B agglutinins of a pregnant woman may rise if the infant possesses the corresponding agglutinin. This has been confirmed by many authors (43), (6), (44), (45) since that time. Smith (43) found a rise in 40 out of 46 heterospecific pregnancies and also made the important observation that mothers were only immunized by infants who secreted A or B blood group substance in their

tissue fluids; such secretion is known to occur in about 80% of Europeans. The relationship of the secretor status of the infant to the rise in maternal isoagglutinin has been confirmed by other workers (46) (47). We did not determine the presence of secretion in the infants of our series. Boorman and her colleagues (6) detected a rise in relevant maternal saline-agglutinin titre in 36 out of 44 cases of heterospecific pregnancy; they noted that the titre increased from 4 to over 64 times and that the peak was usually reached between the tenth and twentieth days of the puerperium. In our series a significant rise in saline anti-A or anti-B agglutinin titre at some stage of heterospecific pregnancy was demonstrated in slightly less than one-half of all cases (20 of 47 women), and it was also found that the maximum was reached more than one week after delivery. When the titre changes were analyzed further it was shown that the bulk of the rise tended to occur in the puerperium, but a slight upward trend was often noted on repeated prenatal testing. We found a rise by at least two dilutions in 4 out of 21 heterospecific pregnancies in which multiple prenatal titrations were performed. A few similar cases are quoted by Smith (43), while other workers have found no significant increases up to the time of delivery (47). This point is of interest in relation to the question of the antigenic stimulus which causes the rise in maternal isoagglutinin titre; if this rise is indeed related to the secretor status of the infant, one may presume that it is due to specific soluble substance secreted by the baby and passing through the placenta. A marked rise in maternal titre occurring during the pue-

perium would suggest that this substance reaches the mother chiefly during labour; the earlier rise in some cases, however, indicates that the stimulus may occasionally occur before delivery.

In homospecific pregnancy, most authors have found no rise in maternal saline-agglutinin titre. Thus Smith (43) found that it occurred only once in 154 homospecific pregnancies, and Zuelzer and Kaplan (47) encountered a slight rise of both anti-A and anti-B after delivery in only 2 of 92 homospecific pregnancies. Yet inspection of Charts 1 and 2 in the paper of the latter authors shows a general slight trend for the titres of anti-A and anti-B in saline to rise after birth even in homospecific cases. Our material also suggests this, both from the median saline titres before and after delivery and also from a detailed analysis of the titre changes, although the rise is not statistically significant. The explanation of this slight rise after delivery in homospecific pregnancy is not clear, but it presumably represents an anamnestic phenomenon caused by stimulation of maternal antibody production by other fetal antigens. The trend is distinctly less marked than in heterospecific pregnancy, yet it is just demonstrable.

The titres in saline and their changes during pregnancy did not differ significantly in primigravidae and multigravidae, either in homospecific or in heterospecific pregnancy. This is in agreement with the findings of Zuelzer and Kaplan (47), while Polayes and McNally (44) found a slightly higher level of saline-agglutinins in multiparous as compared to nulliparous women. On long range postpartum follow-up Zuelzer and Kaplan

(47) found in a few cases that the response of agglutinin titre was subsiding, but had not yet completely disappeared as late as seven months after delivery. Wiener and his colleagues have reported that a high titre may occasionally drop slowly over the course of years (19).

The agglutinin titre in albumin at 37° is difficult to evaluate and shows somewhat similar trends as the titre in saline at room temperature. However, if it exceeds that latter titre by two or more dilutions the presence of an immune response may be presumed. Yet such an "immune" antibody may have been present before the onset of pregnancy and may not harm the infant. In regard to anti-A, this phenomenon of a relatively high titre of agglutination in albumin was present prenatally in 6 of 36 homospecific pregnancies, and persisted into the postpartum period in three of them.

Jouvencaux (48) described a *homospecific* pregnancy in which an immune anti-A, demonstrated by high thermal optimum, even increased in titre during pregnancy. While such evidence of immune ABO antibodies may be encountered in homospecific pregnancies and may not harm the infant even in heterospecific cases, due note should be taken of it. In our 54 heterospecific pregnancies, an excess of the titre in albumin at 37° over that in saline at room temperature by two or more dilutions at some stage before or after birth was found eleven times (8 O-A, 3 O-B) and in two of these the infant subsequently proved to have hemolytic disease.

As regards hemolysins, Jonsson (10) first showed on a large scale that these antibodies tended to appear in maternal

sera as a result of heterospecific pregnancy. Recently anti-A and anti-B hemolysins were investigated carefully by Jungwirth (40) in 500 specimens of pregnant O women and 50 specimens of group B women, as well as in 500 group O men. He concluded that about 75 % of group O pregnant women or male hospital patients had anti-A or anti-B hemolysins in their sera, as compared to 50 % of group O male donors. These figures correspond closely to those which we obtained in pregnant women and blood donors, respectively. It is also of interest that this author found no relation between hemolysin manifestation and the number of previous normal pregnancies. However, our findings are at variance with his in regard to the effects of pregnancy on hemolysin activity; he found very little titre change during pregnancy and sometimes diminution of hemolysins towards the end of pregnancy, while we found a slight increase of anti-A hemolysin activity in some cases of homospecific pregnancy and a marked increase in heterospecific pregnancy, so that all of 23 maternal sera in the latter group were eventually positive for anti-A hemolysin in the postpartum period. Crawford and his colleagues (9) (41) have stressed the usefulness and ease of hemolysin determination, but in heterospecific pregnancy their presence must be regarded as an expected finding. The use of fetal cells obtained from cord blood was therefore suggested (41) in order to show whether the hemolysins in a particular case would be liable to damage the infant's red cells. The test has been used in this manner (47) (49) and fewer positives are evidently obtained with this technique. These results were confirmed in our study.

Incomplete agglutinins and sensitizing antibodies were demonstrated by residual agglutination in albumin and by persistent positive indirect antiglobulin test against A<sub>1</sub> or B cells after neutralization of saline-agglutinins, in many women in our series. Both forms of immune anti-A were present together in about one-third of homospecific pregnancies and singly one of them was positive in another one-fifth, leaving less than half of the women negative for both. The proportions of positives were slightly, but not significantly, higher in heterospecific pregnancies. It is well known that such immune antibodies may be present in maternal sera to a considerable titre without harming the infant (32); and they may also be found in homospecific pregnancy (8). Yet such antibodies merit attention as they are potentially liable to harm an incompatible infant and have been shown to traverse the placenta more easily than saline-agglutinins. Zuelzer and Kaplan (47) state that they never observed anti-A or anti-B immune antibody to develop *de novo* in the course of heterospecific pregnancy, but in our series this occurred three times (Case Nos. 6, 18 and 21)<sup>1</sup> in 54 heterospecific pregnancies.

### Summary

After preliminary serological investigation of 109 male and 63 female random group O blood donors a systematic prospective study of serological findings in 154 group O women attending a Prenatal Clinic was established. They were subsequently found to be divisible into 100 homospecific and 54 heterospecific pregnancies. The levels and changes of dif-

<sup>1</sup> See Part II, Table 15.

ferent forms of anti-A in 49 and of anti-B in 20 homospecific pregnancies were compared to those of the incompatible maternal antibodies in the heterospecific pregnancies.

The titres of anti-A and anti-B agglutinins in saline at room temperature were found to cover a wide range and to be approximately equal initially in homospecific and heterospecific pregnancies and in primigravidae and multigravidae.

In homospecific pregnancy the titres in saline showed a slight rise, presumably of an anamnestic nature, after delivery in a small proportion of mothers (10 out of 49, or 20.0% in the case of anti-A). In heterospecific pregnancies this postpartum rise of titre was considerably more marked and frequent, occurring to a significant degree in 14 out of 34 of O-A cases; (41.1%) in some of these a slight rise was beginning to occur during pregnancy.

Titres of anti-A and anti-B agglutinins in albumin at 37°C followed a somewhat similar pattern. Prenatally for anti-A, this titre exceeded that of saline agglutination by two or more dilutions in 6 of 36 homospecific and 5 of 37 heterospecific O-A pregnancies, thus suggesting the presence of an incomplete agglutinin. Postnatally for anti-A, such a difference in titres was found in 5 of 39 homospecific and in 8 of 36 heterospecific O-A pregnancies.

Anti-A hemolysins were found in about three-quarters of homospecific and O-A heterospecific pregnancies. After delivery their incidence rose to 37 out of 43 (81%) homospecific and to all of 23 (100%) heterospecific pregnancies; the changes in anti-B hemolysins were less marked. When tests for maternal hemolysins were performed against fetal instead of adult A<sub>1</sub>

cells as recommended by Crawford and colleagues (41), hemolysis was usually less marked and high titres (8 or more) were more significant in terms of possible risk to the infant.

Residual agglutination in albumin and a positive indirect antiglobulin test against A cells were demonstrated in the maternal sera in about one-third of homospecific pregnancies and in a slightly, but not significantly, larger proportion of heterospecific cases. These antibodies were also found singly in another one-fifth of the mothers, so that less than one-half of the women had neither form of immune anti-A. In 3 of 54 heterospecific pregnancies such immune anti-A arose *de novo* after delivery. The titres and incidence of these immune antibodies did not change markedly postpartum but there was a trend for increased amounts of group specific substance to be required for neutralization, particularly in heterospecific pregnancy.

During pregnancy about one-fifth of the women in the homospecific and one-third in the heterospecific group had all three forms of immune anti-A (hemolysin, positive indirect antiglobulin test after neutralization and residual agglutination in albumin) and only about one-sixth of each group were negative for all these tests while the rest appeared "partially immune", commonly owing to the presence of hemolysins. After delivery there was a slight change towards a higher incidence of immune antibodies in homospecific pregnancy and a more definite one in heterospecific cases where 9 of 23 mothers eventually had all three forms of immune anti-A and all had the anti-A hemolysin.

The findings are discussed in relation to those of other workers.



PART II

The Transfer of Maternal Anti-A and Anti-B  
Antibodies to the Infant

*by*

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## PART II

### The Transfer of Maternal Anti-A and Anti-B Antibodies to the Infant

In the first section of this paper the levels of maternal anti-A and anti-B antibodies and their changes during pregnancy and in the postnatal period were described in a series of group O women in homospecific and heterospecific pregnancy. In this section the serological findings in the cord blood of the infants will be reviewed and compared with those of the mother.

The placental permeability for maternal anti-A and anti-B saline-agglutinins in different types of blood group relationship between mother and infant were first examined thoroughly by Hirszfeld and Zborowski in 1925 (16). They reached the following important conclusions:

1. Cord blood contains no agglutinins formed by the infant, and any ABO agglutinins present in it are derived from the mother.

2. When iso-agglutinins are present in the serum of the newborn their level is lower than in the mother (maximally  $\frac{1}{4}$  of the maternal level).

3. Iso-agglutinins pass into the fetus in different amounts according to the blood group relationship; thus they were demonstrable in over 90 % of the infants in homospecific O-O pregnancy, but in only 22 % of homospecific B-B and in only 7.5 % of A-A cases. In heterospecific pregnancy the passage of incompatible

saline-agglutinins could rarely be demonstrated at all. For instance, in the O-A relationship the authors found significant amounts of anti-A in the cord blood in only one out of 26 cases, and in the O-B relationship anti-B was not detected in any of 11 cord blood specimens.

These results have been generally confirmed by other workers. It has also been observed that the ratio between maternal and fetal agglutinin levels shows considerable variation from case to case, even with the same blood group relationships. In homospecific pregnancies there has been no evidence to support the possibility that a high titre of maternal iso-antibodies would lead to an easier overflow of agglutinin into the cord blood (46). When incomplete ABO agglutinins became recognized their passage through the placenta was also studied, and Wiener (17) showed that these antibodies (which he called univalent and designated as "glutinins") traversed the placenta more readily than saline-agglutinins (which he called bivalent). He concluded that the incomplete agglutinins were probably comprised of smaller molecules than the saline-agglutinins. There is some disagreement in the literature concerning the proportion of cases of homospecific pregnancy in which the saline-agglutinin reaches the cord blood, but with modern techniques

they can be demonstrated in the great majority of cases (1) (50). Nonetheless, it is generally agreed that incomplete agglutinins penetrate in relatively larger amounts as Wiener has suggested (17) (7). This need not simply be due to the filtering action of the placenta on the larger molecules of the saline-agglutinin, but rather the placental or fetal tissues may neutralize the maternal agglutinins by group specific substances which are known to be more effective against the saline than the incomplete type of agglutinin. This concept is supported by the finding that in O-A or O-B heterospecific pregnancy the agglutinin antagonistic to the fetal blood group is nearly always absent from the cord serum, whereas the heterologous antibody may be found in the cord sera with nearly the same frequency as in homospecific pregnancy (50). No evidence has been found that the secretor property in the infant reduces the amount of incompatible maternal antibody reaching the baby, and it is postulated that the placental and fetal tissue antigens are chiefly effective in neutralizing incompatible agglutinins (50).

Incidentally, in homospecific O-O pregnancy saline anti-B agglutinin usually has a lower titre than anti-A in the fetal circulation; this may be due partly to diminished placental permeability but it also may be due to the fact that in the maternal serum the average titre of saline anti-B agglutinin is lower than that of anti-A (51) (50). Similarly, the incidence of anti-A hemolysins in O persons is higher than that of anti-B hemolysins (14).

In regard to hemolysins it is not yet certain how often these penetrate the placenta. Seelen (51) demonstrated them

in the cord serum in only 15 out of 60 homospecific pregnancies in which they were found in the maternal serum. Jungwirth (40) demonstrated placental transfer of hemolysins in 10 out of 17 homospecific pregnancies, but did not detect even a compatible maternal hemolysin in the cord blood in 9 heterospecific pregnancies. Both authors considered that the hemolysins passed the placenta more easily in homospecific pregnancy when the maternal serum contained other immune antibodies.

The placental passage of sensitizing antibodies does not appear to have been investigated fully as yet (40); it is not known for certain what proportion of cord bloods give a positive indirect antiglobulin test against A or B cells in homospecific and heterospecific pregnancy. In 1956, Gunson and Goodfellow (52) stated that they had found the test consistently negative in 25 normal group A or B infants born to group O mothers. Subsequently Gunson (53) extended these findings. In 115 normal unaffected group A or B infants with group O mothers, he found that the indirect antiglobulin test performed on cord blood was consistently negative. However, in a clinically affected group who developed jaundice in the first 24 hours of life, the indirect antiglobulin test was positive in 39 out of 42 (93%) infants. The direct antiglobulin test is, of course, negative in the cord blood of homospecific infants in the absence of other blood group incompatibility; in heterospecific pregnancies it has occasionally been found to be positive when large series were tested. Thus, among 209 Rh-compatible pregnancies with ABO incompatibility Jakobowicz and colleagues (54) found three in-

stances of a positive direct antiglobulin test in the cord blood. Rosenfield (49), using a very sensitive technique, found a weakly positive direct antiglobulin test in the cord blood of over 17% of group-incompatible infants; 38 of the 39 mothers of such incompatible infants belonged to group O. In Gunson's (53) clinically affected group the direct antiglobulin test was weakly positive in 16 out of 42 infants (39%) during the first 24 hours, while the test was uniformly negative in 115 unaffected babies derived from heterospecific pregnancy.

### Present Study

In the present work the prenatal serological findings in the mother are related to the cord findings in the offspring of 69 O-O homospecific, and 40 O-A and 14 O-B heterospecific pregnancies. The study resembles that of Zuelzer and Kaplan (50) although somewhat different serological methods were used. Investigations in the mother included anti-A and anti-B titrations in saline at room temperature and in albumin at 37°C, and the performance of an indirect antiglobulin test against A<sub>1</sub> or B cells and the estimation of residual agglutination in albumin after complete neutralization of saline-agglutinin with group specific substance. Maternal anti-A and anti-B hemolysins were also investigated. In the infant's cord blood the amounts of anti-A or anti-B agglutinin in saline and albumin were estimated similarly; the direct antiglobulin test was performed in all infants while the indirect test was only applied in heterospecific cases.

### Results

I. *Homospecific Pregnancies.* There were 69 O-O pregnancies in this group, with 70 infants, owing to one twin birth. Anti-A studies were performed in 49 and anti-B in the remaining 20. The serological findings in mother and cord are shown in Tables 13 and 14.

(1) *Agglutinins in Saline and Albumin.* The presence of saline anti-A agglutinin was determined in 45 infants, with 41 (91%) positive results. Agglutinin acting in albumin at 37° was demonstrated in 34 out of 39 (87%) cord specimens. In the 5 cases in whom the presence of saline-agglutinin in cord blood was not determined, the tests for agglutinins in albumin were also omitted. In the 4 in whom no saline-agglutinin was demonstrable, agglutinin in albumin was also absent in 2 and was not tested in the other two.

Saline/albumin (S/A) ratios in maternal and cord blood indicating the relative strength of these two titres in each case are also tabulated in Table 13. It is clear that the median of this ratio is higher in the maternal than in the cord blood. In other words, although both forms of agglutinin pass the placenta in homospecific pregnancy, in the great majority of all cases the titre of the saline-agglutinin is diminished more during the passage than that of agglutinin in albumin. In the maternal blood the S/A ratio varies from 32/1 to 1/16 with a median value of 1:1, whereas in the cord blood it varies from 8/1 to 1/64 with a median value of 1:4.

In the mother, only 6 out of 36 sera showed a two tube excess of titre in albumin over that in saline (Table 13, Case Nos. 20, 28, 29, 36, 38 and 40), whereas in the infants such an excess

TABLE 13. *Prenatal maternal and infant cord serological findings in 49 homospecific (O-O) pregnancies with regard to anti-A antibodies. (D- denotes absence of D factor, NT - not tested, D+ denotes presence of D factor.)*

Prenatal maternal serology										Infant cord serology					
Case no.	Parity	Rh type	Agglutinin titre			After neutralization				Agglutinin titre			Direct AHG test		
			Saline	Albumin	S/A ratio	Hemolysin	Parts to neutralize*	Incomplete agglutinin	Indirect AHG test	Rh type	Saline	Albumin	S/A ratio	Commercial serum	Dilutions of native serum
O-O															
1	3	D +	2560	320	8:1	—	2	—	—	D +	64	32	2:1	—	—
2	1	D +	1280	NT	—	—	4	—	—	D +	NT	NT	—	—	—
3	1	D —	640	320	2:1	8	12	—	+	D +	32	256	1:8	—	—
4	7	cde/cde	640	80	8:1	4	4	—	+	D —	80	Nil	—	—	—
5	1	cde/cde	320	160	2:1	4	2	—	—	D —	20	20	1:1	—	—
6	1	D +	160	80	2:1	—	3	32	—	D +	4	4	1:1	—	—
7	1	D +	160	160	1:1	NT	9	—	—	D +	128	128	1:1	—	—
8	6	D +	80	160	1:2	—	9	—	+	D +	Nil	NT	—	—	—
9	5	D +	80	80	1:1	—	3	—	—	D +	Nil	Nil	—	—	—
10	8	D +	2560	1280	2:1	8	9	—	—	D +	32	1024	1:32	—	—
11	1	D +	1280	640	2:1	4	9	—	—	D +	8	16	1:2	—	—
12	1	D +	640	NT	—	—	3	—	—	D +	Nil	Nil	—	—	—
13	1	D +	640	NT	—	2	7	—	—	D +	10	NT	—	—	—
14	3	D +	320	640	1:2	NT	4	80	+	D +	16	256	1:16	—	—
15	5	D +	160	320	1:2	4	9	80	—	D +	64	128	1:2	—	—
16	4	D +	320	NT	—	4	9	—	—	D +	80	NT	—	—	—
17	1	D +	640	640	1:1	4	3	—	—	D +	256	32	8:1	—	—
18	4	D +	640	NT	—	4	9	—	+	D +	NT	NT	—	—	—
19	1	D —	320	NT	—	2	2	—	—	cde/cde	Nil	NT	—	—	—
20	1	D +	320	1280	1:4	8	6	—	—	D +	32	64	1:2	—	—
21	4	D +	640	NT	—	4	9	80	+	D —	64	256	1:4	—	—
22	7	D +	160	NT	—	2	3	16	—	D +	64	NT	—	—	—
23	1	D +	1280	640	2:1	8	9	—	—	D +	4	32	1:8	—	—
24	2	D +	320	320	1:1	4	9	—	—	D +	32	32	1:1	—	—
25	7	cde/cde	40	40	1:1	—	2	—	—	D —	4	32	1:8	—	—
26	1	D +	5120	NT	—	2	9	80	+	D +	32	Nil	—	—	—
27	8	D +	1280	640	2:1	8	3	—	—	D +	16	16	1:1	—	—
28	3	D +	1280	20,480	1:16	4	27	—	+	D +	32	1024	1:32	—	—
29	1	D +	320	1280	1:4	8	15	—	—	D +	128	1024	1:8	—	—
30	1	D +	2560	2560	1:1	16	12	80	+	D +	64	128	1:2	—	—
31	1	D +	320	NT	—	4	9	—	—	D +	NT	NT	—	—	—
32	1	D +	160	NT	—	2	2	12	—	D +	NT	NT	—	—	—
33	1	D +	320	320	1:1	8	15	—	—	D +	32	64	1:2	—	—
34	1	cde/cde	320	NT	—	8	3	32	+	D —	160	NT	—	—	—
35A	5	D +	640	NT	—	4	4	40	+	D +	40	Nil	—	—	—
B															
36	10	D +	80	640	1:8	NT	4	40	+	D +	8	64	1:8	—	—
37	2	D +	640	640	1:1	8	9	40	+	D +	32	512	1:16	—	—
38	1	D +	320	1280	1:4	8	9	80	—	D +	16	1024	1:64	—	—
39	2	D +	320	640	1:2	NT	9	40	+	D +	4	16	1:4	—	—
40	5	cde/cde	20	80	1:4	4	4	—	—	cde/cde	2	8	1:4	—	—
41	2	D +	10,240	320	32:1	8	21	—	—	D +	2	32	1:16	—	—
42	6	cde/cde	5120	160	32:1	4	9	16	+	cde/cde	64	64	1:1	—	—

\* Amount of A substance required for complete neutralization of standard amount of serum.

Table 13 (cont.).

Prenatal maternal serology										Infant cord serology						
Case no.	Parity	Rh type	Agglutinin titre			After neutralization				Agglutinin titre			Direct AHG test			
			Saline	Albumin	S/A ratio	Hemolysin	Parts to neutralize	Incomplete agglutinin	Indirect AHG test	Rh type	Saline	Albumin	S/A ratio	Commercial serum	Dilutions of native serum	
43	1	D +	2560	1280	2:1	8	9	32	+	D +	64	512	1:8	-	-	
44	2	cde/cde	160	320	1:2	4	3	128	+	D +	16	128	1:8	-	-	
45	1	cde/cde	5120	640	8:1	4	9	320	+	cde/cde	32	128	1:4	-	-	
46	1	D +	320	640	1:2	8	21	16	+	D +	8	128	1:16	-	-	
47	2	cde/cde	80	80	1:1	4		NT	NT	cde/cde	64	128	1:2	-	-	
48	2	D +	640	160	4:1	4	9	-	-	D +	64	256	1:4	-	-	
49	2	cde/cde	320	640	1:2	4	9	80	+	cde/cde	64	2048	1:32	-	-	

was found 20 times among 34 specimens in which both forms of antibody were found. To put this in different words, the maternal/fetal ratio of the titres in these two forms of antibody was generally greater for saline-agglutinins than for agglutinins in albumin. All this is in accord with the findings of previous workers.

There is no clear quantitative relationship between the relative titres of agglutinin in saline and albumin as compared between mother and infant. However, it may be noted that the maternal titre in saline always exceeds that of the baby. On the other hand, in 3 out of 34 cases the cord titre of agglutinin in albumin exceeds that of the mother (Table 13, Case Nos. 47, 48, 49). These titres must not be considered as highly accurate because pseudo-agglutination may occur when serum from cord blood is diluted in bovine albumin, but when agglutination of group A or B cells was stronger than that of control group O cells the result was taken as being genuinely due to

specific agglutination. It may appear paradoxical that the cord titre should ever exceed the maternal one, and this may indeed be due to limited accuracy of the method, but Jungwirth (40) encountered the same phenomenon in regard to sensitizing antibody and pointed out that bacterial antibodies are also known to reach higher titres in the cord than in the maternal blood.

The corresponding results of studies of anti-B titres in 20 homospecific pregnancies are shown in Table 14. They show a similar trend, although the maternal titres are lower, and the cord levels of agglutinin in albumin never exceeded those of the maternal serum.

There appear to be no significant differences in regard to the levels and changes of these anti-A and anti-B agglutinins as between the primigravidae and multigravidae.

(2) *Direct Antiglobulin Test.* The direct antiglobulin test was negative in the cord blood of all 70 infants, using both com-

TABLE 14. *Prenatal maternal and infant cord serological findings in 20 homospecific (O-O) pregnancies with regard to anti-B antibodies. (NT = not tested, D+ denotes presence of D factor.)*

Case no.	parity	Prenatal maternal serology					Infant cord serology					Direct AHG Test				
		Rh type	Agglutinin titre		S/A ratio	Hemo-lysins	Parts to neu-tralize*		After neutralization		Rh type	Agglutinin titre		S/A ratio	Commer-cial serum	Dilu-tions of native serum
			Saline	Albumin			Incomplete agglutinin	Indirect AHG test	Saline	Albumin						
1	1	D +	5120	1280	4:1	NT	2	24	+	D	64	32	2:1	—	—	
2	1	D +	5120	1280	4:1	NT	2	nil	+	D	16	32	1:2	—	—	
3	6	D +	1280	5120	1:4	NT	2	nil	+	D	4	32	1:8	—	—	
4	3	D +	2560	640	4:1	NT	4	20	+	D	2	4	1:2	—	—	
5	5	D +	40	40	1:1	NT	2	—	+	D	2	4	1:2	—	—	
6	5	D +	640	640	1:1	NT	7	—	+	D	8	16	1:2	—	—	
7	4	D +	320	640	1:2	4	9	40	—	D	32	64	1:2	—	—	
8	1	D +	160	80	2:1	NT	9	—	+	D	32	32	1:1	—	—	
9	9	D +	320	160	2:1	4	9	80	—	D	8	4	2:1	—	—	
10	1	D +	160	160	2:1	2	9	—	—	D	1	2	1:2	—	—	
11	2	D +	320	160	2:1	NT	12	—	—	D	64	32	2:1	—	—	
12	1	D +	320	160	2:1	NT	7	32	—	D	32	128	1:4	—	—	
13	1	D +	80	80	1:1	NT	4	16	—	D	2	4	1:2	—	—	
14	2	D +	320	80	4:1	NT	7	—	+	D	4	16	1:4	—	—	
15	3	D +	320	160	2:1	NT	3	32	+	D	16	64	1:4	—	—	
16	1	ede/cde	160	320	1:2	NT	4	20	—	D	16	32	1:2	—	—	
17	5	D +	20	20	1:1	NT	2	12	—	D	8	4	2:1	—	—	
18	4	D +	80	80	1:1	NT	2	—	—	D	4	4	1:1	—	—	
19	6	ede/cde	640	160	4:1	4	4	80	+	D	0	2	0:2	—	—	
20	8	D +	80	40	2:1	NT	2	24	—	D	0	8	0:8	—	—	

\* Amount of B substance required for complete neutralization of standard amount of serum.

mercial serum and various dilutions of native antiglobulin serum.

II. *Heterospecific Pregnancies.* Serological comparisons were made between prenatal and cord samples and the results are shown in Table 15. There were 54 pregnancies (40 O-A and 14 O-B), and owing to one twin delivery, 55 infants resulted.

(1) *Agglutinins in Saline and Albumin.* In this group, saline anti-A agglutinins in group A babies and saline anti-B agglutinins in group B babies were rarely demonstrable. The anti-A agglutinins were only encountered in low titre in 2 out of 41 group A infants and anti-B agglutinins in 1 out of 14 group B babies. One of these 3 infants exhibited a positive direct antiglobulin test in the cord blood. The titres of saline-agglutinins are only 2 to 4, and Wiener has pointed out that such low titre agglutinations in saline may be due to incomplete agglutinins, as the infant's serum is relatively undiluted and provides a colloid medium (7) (27).

On the other hand, the incompatible maternal agglutinin was demonstrable in albumin at 37° in 28 out of 46 cord bloods tested. Its titre was generally low, though in one specimen it reached 64. (It is of interest that this infant had a positive direct antiglobulin test in the cord blood.) The level of antibody was always lower than in the maternal serum, and the maternal/fetal ratio of this titre was quite often high, ranging from 320/32 to 5120/1. Again rouleaux were frequently noted in this titration, and control agglutination against group O cells was always set up.

(2) *The Indirect Antiglobulin Test.* The indirect antiglobulin test against adult A<sub>1</sub> cell in the case of group A infants, or

adult B cells in the case of group B infants, was performed using cord serum. In 3 cases the presence of saline-agglutinin interfered with the performance of the tests. It was therefore omitted in two of these, while in the third the saline-agglutinin was neutralized and the test then proved positive. Among the remaining 52 cord specimens the indirect antiglobulin test was positive in 24 and negative in 28 cases. Thus the test is known to have been positive in 25 out of 53 infants.

The results of the indirect antiglobulin test in the mother (after neutralization) and the infant are compared in Fig. 3. The test was positive in 28 mothers prenatally; and the sensitizing antibody was demonstrated in 15 of their offspring and was not tested in two. The test was negative in 25 mothers, but sensitizing antibody was demonstrated in 9 of their offspring. The negative findings in these 9 mothers must presumably be due to inadequacy of the technique involving neutralization ("serological failures"). One further mother

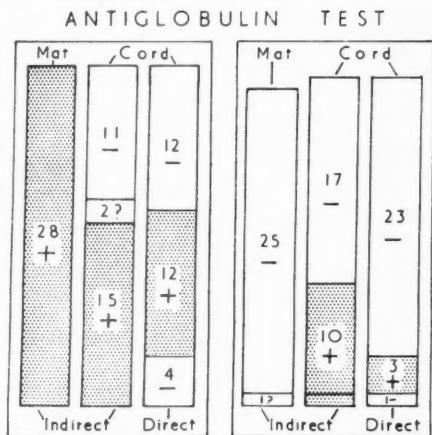


Fig. 3. Relationship of prenatal maternal and cord antiglobulin tests in 54 heterospecific pregnancies.

TABLE 15. *Prenatal maternal and infant cord serological findings in 54 helio- refer to the isoantibody antagonistic to the infants ABO blood group (D)-*

Prenatal maternal serology							After neutralization		
Case no.	Parity	Rh type	Agglutinin titre		Hemolysin	Parts to neutralize***	Incomplete agglutinin	Indirect ABO type	
			Saline	Albumin					
O-A. Negative direct anti-human									
1	1	D +	160	160	—	3	Nil	—	
2	1	D +	2560	1280	4	3	Nil	—	
3	1	D +	640	160	4	3	Nil	—	
4	1	D +	80	80	2	3	Nil	—	
5	1	D +	320	320	4	3	Nil	—	
6	1	cde/cde	40	40	—	2	Nil	—	
7	1	D +	1280	640	—	3	Nil	—	
8	3	D +	2560	320	4	3	Nil	—	
9A	7	D +	320	NT	—	3	Nil	—	
B									
10	4	D +	1280	640	4	3	Nil	—	
11	1	cde/cde	160	80	4	3	32	—	
12	1	D +	2560	160	8	4	Nil	—	
13	2	D +	320	320	2	2	Nil	—	
14	9	cde/cde	320	80	4	9	40	—	
15	3	D +	NT	NT	NT	NT	NT	—	
16	1	D +	640	320	2	2	24	—	
17	2	D +	40	80	NT	2	24	—	
18	3	cde/cde	80	80	NT	2	Nil	—	
19	1	D +	320	320	NT	2	12	—	
20	1	cde/cde	80	40	8	4	20	—	
21	2	D +	160	320	8	3	Nil	—	
22	5	D +	5120	5120	8	6	Nil	—	
23	2	D +	640	1280	4	4	Nil	—	
24	1	cde/cde	320	320	NT	3	32	—	
25	1	D +	80	320	4	2	Nil	—	
26	1	D +	320	2560	8	3	64	—	
27	1	D +	320	2560	16	15	12	—	
O-B. Negative direct anti-human									
28	1	D +	160	80	2	4	40	—	
29	1	D +	320	160	8**	54	80	—	
30	1	D +	160	40	NT	9	40	—	
31	1	D +	160	40	8**	9	20	—	
32	1	D +	20	160	NT	2	24	—	
33	7	D +	160	320	8**	3	128	—	
34	2	cde/cde	320	320	NT	9	80	—	
35	8	D +	80	80	2**	4	40	—	
36	7	D +	20	20	4**		NT	NT	
37	4	cde/cde	40	40	Neg	2	Nil	—	
38	2	cde/cde	1280	320	4**	9	80	—	
39	1	D +	640	2560	4**	57	80	—	

\* 1 day postnatal.

\*\* Anti-A lysin.

\*\*\* Amounts of A or B substance required for complete neutralization of standard amount of serum

specific (40 O-A and 14 O-B) pregnancies. Unless otherwise indicated the results denote absence of D factor, NT = not tested, D+ denotes presence of D factor.)

## Infant cord serology

Indirect AHG type		Direct AHG test							Indirect AHG test
		Agglutinin titre		Comm. serum	Dilutions of native serum				
Saline	Albumin	1/10	1/50		1/100	1/200	1/500		
anti-human albumin test in infant									
-	NT	-	-	-	-	-	-	-	
-	NT	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	NT	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	4	-	-	-	-	-	-	-	
-	2	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	R	-	-	-	-	-	-	-	
-	1	-	-	-	-	-	-	-	
-	2	-	-	-	-	-	-	-	
-	2	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	R	-	-	-	-	-	-	-	
-	2	-	-	-	-	-	-	-	
-	1	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	32	-	-	-	-	-	-	-	
-	2	-	-	-	-	-	-	-	
-	8	-	-	-	-	-	-	-	
-	8	-	-	-	-	-	-	-	
anti-human albumin test in infant									
-	NT	-	-	-	-	-	-	-	
-	NT	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	
-	4	-	-	-	-	-	-	-	
-	R	-	-	-	-	-	-	-	
-	4	-	-	-	-	-	-	-	
-	R	-	-	-	-	-	-	-	
-	2	-	-	-	-	-	-	-	
NT	2	-	-	-	-	-	-	-	
side	2	-	-	-	-	-	-	-	
side	-	-	-	-	-	-	-	-	
-	2	-	-	-	-	-	-	-	

after neutralization.

Omitted, rouleaux formation.

Table 15 (cont.).

Prenatal maternal serology								
Case no.	Parity	Rh type	Agglutinin titre		Hemolysin	Parts to neutralize	After neutralization	
			Saline	Albumin			Incomplete agglutinin	Indirect Agglutination type
<i>O-A. Positive direct anti-human agglutinin tests</i>								
40	1	D +	160	NT	—	9	8	D +
41	3	D +	1280	2560	4	2	48	D +
42	5	CDe/CDe	1280	320	2	2	24	D +
43	1	D +	160	40	NT	3	Nil	D +
44	8	D +	160	320	16	3	Nil	D +
45	9	D +	320	160	NT	2	24	D +
46	1	cde/cde	640	320	2	9	20	D +
47	1	D +	160	640	—	9	640	D +
48	1	cde/cde	10240	5120	8	9	320	D +
49	2	D +	160	160	4	4	160	D +
50	3	CDe/cde	1280	5120	8	6	Nil	D + cde
51	1	D +	640	320	4	2	12	D +
52	4	D +	160	40	NT	2	48	D +
<i>O-B. Positive direct anti-human agglutinin tests</i>								
53	2	D +	640	640	NT	2	Nil	D +
54	2	D +	640	5120	4	45	256	D +

did not have the test performed before birth and had an infant with a positive indirect antiglobulin test. Assuming that sensitizing antibody in the infant must have been derived from the mother, we may conclude that at least 38 out of 54 mothers (70%) in this heterospecific series must have had sensitizing antibody in their serum during the prenatal period.

It is interesting that agglutination of adult cells of the infant's group in albumin by the cord sera was also demonstrable in 17 out of the 25 babies with positive indirect antiglobulin test and was doubtful in two cases which showed rouleaux formation.

(3) *The Direct Antiglobulin Test.* In 15 out of 55 infants tested the direct antiglobulin test was positive in cord blood. Table 16 shows that it was only positive

TABLE 16. *Analysis of direct anti-human globulin (AHG) tests, using commercial and a potent native serum, on cord blood in infants born of heterospecific pregnancy.*

	No. of cases tested	Test positive	Test negative
Comm. AHG Serum	15	7	8
Dilutions of Potent	1:10 15	1	14
	1:50 15	8	7
Native AHG	1:100 15	15	0
Serum	1:200 15	12	3
	1:500 14	6	8

in 7 cases<sup>1</sup> when commercial antiglobulin serum was used, but that the titration method with a potent native antiglobulin serum gave 15 positive results at a dilution

<sup>1</sup> In 1 further case it was negative in cord blood but positive in 2 subsequent tests during the first 48 hours.

Infant cord serology									
Indirect AHG type	Direct AHG Test								Indirect AHG test
	Agglutinin titre		Comm. serum	Dilutions of native serum					
	Saline	Albumin			1/10	1/50	1/100	1/200	1/500
<i>Anti-human albumin test in infant</i>									
D-	4	64	-	+	+	+	-	-	NT
D-	-	4	+	-	-	+	+	+	+
D-	-	-	+	-	-	+	+	NT	+
D-	-	-	+	-	+	+	+	+	+
D-	-	4	-	-	-	+	+	-	+
D-	-	8	-*	-	-	+	+	-	+
D-	-	4	+	-	+	+	+	+	+
D-	-	4	-	-	+	+	+	+	+
D-	-	16	-	-	+	+	+	+	+
D-	-	1	+	-	+	+	+	+	+
Rede	-	8	+	-	+	+	+	+	+
D-	-	-	-	-	-	+	-	-	+
D-	-	2	+	-	+	+	-	-	+
<i>Anti-human albumin test in infant</i>									
D-	-	32	-	-	-	+	+	-	+
D-	-	1	-	-	-	+	+	-	+

\*Positive in 2 subsequent tests.

of 1:100. The advantage of using titrated potent antiglobulin serum is thus evident. It is also important to test cord specimens, or if these are not available blood specimens from the infant as soon as possible after birth, as the test does not remain positive for long in most cases, particularly when commercial serum is used. This is shown well in three of our infants who had repeated direct antiglobulin tests done after birth, as shown in Table 17. It is clear that both the direct and the indirect antiglobulin test, if initially positive, tend to become negative within the first few days after birth.

In the 15 cases with positive direct antiglobulin tests the indirect test using cord serum and adult homologous cells was positive in 14, and in the remaining one there was saline anti-A agglutinin

which interfered with the performance of the test. Thus the direct antiglobulin test appears to be accompanied by a positive indirect test as might be expected. The converse is not true as the indirect antiglobulin test merely indicates the presence of sensitizing antibody in the serum, but not necessarily the fixation of this antibody on the baby's red cells.

Anti-A or anti-B agglutinins demonstrable in albumin at 37° were present in 12 out of the 15 infants with a positive antiglobulin test; thus the correlation between this form of immune antibody and a positive direct antiglobulin test appeared to be slightly less constant than that of the two forms of the antiglobulin test. The correlation between the presence of agglutination in albumin and the antiglobulin tests is evidently only a loose one,

TABLE 17. *Results of serial direct anti-human globulin (AHG) tests in three infants in heterospecific pregnancies. (NT = not tested.)*

Case no.	Sex	No. of previous pregnancies	Baby's group and Rh type	Age at test	Direct AHG test						Infant's indirect AHG test vs. adult homologous cells	Infant's agglutination titre vs. homologous cells in albumin		
					Commercial AHG serum	Titration of native AHG serum								
						1/10	1/50	1/100	1/200	1/500				
42	F	4	A	Cord	Pos.	Neg.	Neg.	Pos.	Pos.	NT	Pos.	Nil		
					CDe/CDe	4½ days	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Pos.	Nil
43	F	0	A	Cord	Pos.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Nil		
					cde/cde	30 hrs	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.	Pos.	Nil
					60 hrs	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.	Pos.	NT	
					72 hrs	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.	NT	
					96 hrs	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Weak Pos.	NT	
					14 days	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	NT	
50	F	2	A	Cord	Pos.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	8		
					cde/cde	2 hrs	Pos.	Neg.	Pos.	Pos.	Pos.	Neg.	Pos.	2
					26 hrs	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.	Neg.	2	

but it is striking in our series that an incomplete agglutinin titre of 8 or more in the infant's cord blood was always associated with a positive indirect antiglobulin test (when tested) and in 5 out of 8 cases also with a positive direct antiglobulin test.

(4) *Maternal Immune Status in Relation to Infants' Direct Antiglobulin Test.* A detailed analysis of the various forms of immune antibody in the mother in relation to the direct antiglobulin test in the infant is shown in Table 18. This includes all the cases of heterospecific pregnancy in whom prenatal tests for hemolysins were available as well as the results of the indirect antiglobulin test and residual agglutination in albumin after neutralization. The table shows that a positive direct antiglobulin test in the infant is most likely to be encountered when all three forms of immune ABO antibody are present in the maternal serum but may occur in the absence of demonstrable maternal im-

mune antibodies. The latter cases presumably represent failures of technique as also occurred in other reported series (50). A higher proportion of maternal sera might well be found to give a positive indirect antiglobulin test after neutralization if titrated native antiglobulin serum was used (55). Analyzing each antibody separately one finds the following correlations:

1. In 34 cases in which the maternal serum showed a hemolysin prenatally the infant's direct antiglobulin test was positive 9 times.

2. In 21 cases in which the maternal indirect antiglobulin test after complete neutralization was positive, the infant's direct antiglobulin test was positive 10 times.

3. In 22 cases in which the maternal serum showed residual albumin agglutinins after neutralization, the infant's direct antiglobulin test was positive 9 times.

Thus, in this whole series, the mother's indirect antiglobulin test had the highest

TABLE 18. *Correlation between prenatal "immune" antibodies of mothers and direct antihuman globulin (AHG) test of infants in heterospecific pregnancy in cases in which all three maternal antibody tests were performed.*

Maternal "immune" antibodies			No. of women in each maternal-fetal blood-group relationship	Infant's direct AHG test	
Anti-A or anti-B hemolysin	After Neutralization Indirect AHG test	Incomplete agglutinin		Positive	Negative
			Total	Total	Total
—	—	—	O-A 4 } O-B 0 } 4	0	4 } 4
—	—	+	O-A 0 } O-B 0 } 0	0	0
—	+	—	O-A 0 } O-B 1 } 1	0	0 } 1
+	—	—	O-A 10 } O-B 0 } 10	1 } 0 } 1	9 } 0 } 9
+	—	+	O-A 3 } O-B 3 } 6	0	3 } 3 } 6
+	+	—	O-A 4 } O-B 0 } 4	1 } 0 } 1	3 } 0 } 3
—	+	+	O-A 2 } O-B 0 } 2	2 } 0 } 2	0
+	+	+	O-A 9 } O-B 5 } 14	6 } 1 } 7	3 } 4 } 7
Total			O-A 32 } O-B 9 } 41	10 } 1 } 11	22 } 8 } 30

association with a positive direct anti-globulin test in the infant (approximately 2:1). The importance of the maternal indirect antiglobulin test has been stressed previously (56).

### Discussion

Our findings concerning the placental transfer of agglutinins in saline and colloid media in homospecific pregnancy agree with those of Tovey (1) and Zuelzer and Kaplan (50). We found that both types of antibody reached the cord blood in the great majority of cases. Like the latter authors we also found that the titre ratio of a glutinin in saline to that in albumin

was lower in the cord blood than in the maternal serum, and this could well be explained by neutralization of the saline-agglutinin in the placental or fetal tissues. In other words, as Witebsky (32) first suggested, the fetal tissues may act in vivo like the addition of group specific substance in vitro. The titre of agglutinin in albumin at 37° in the cord serum may equal, or rarely even exceed, the maternal titre; though the exact figures are technically unreliable the phenomenon is of interest in analogy to the occasional higher titres of sensitizing antibody in the cord blood found by Jungwirth (40).

In the heterospecific pregnancies the

cord findings are quite remarkably different. Incompatible saline-agglutinins are rarely found and then only in very low titre, and incompatible agglutinins in albumin, found in about two-thirds of the cases, also attain a much lower titre than in homospecific pregnancy. It is of interest that Zuelzer and Kaplan (50) were unable to demonstrate anti-A in saline or gum acacia in 43 of 47 group A infants and anti-B in either medium in any of 53 group B infants; presumably our considerably more frequent finding of incomplete agglutinins must be due to a difference in technique. Three of Zuelzer and Kaplan's group A infants who did have immune anti-A in their serum belonged to sub-group  $A_2$ ; as we did not perform consistent sub-grouping of our infants we cannot state the proportion of  $A_2$  babies in our series.

Jakobowicz and Bryce (46) found an incompatible saline-agglutinin in the infant in 4 out of 79 cases of heterospecific pregnancy (in the present narrow sense of the word). They pointed out that there was no evidence of any hemolytic process in these infants, though one developed "physiological" jaundice. However, in the 3 infants in our series of heterospecific pregnancies who had demonstrable saline-agglutinin in their cord blood, the direct antiglobulin test was positive in one although there was no evidence of clinical "hemolytic disease", as will be discussed later. The presence of incomplete agglutinin in the baby's cord serum to a titre of 8 or more was also a somewhat serious sign as it was associated with a positive direct antiglobulin test in 5 out of the 8 cases concerned.

In our hands the indirect antiglobulin test performed on the infants' serum with

adult homologous cells proved more useful than the search for agglutinin in albumin, though it was positive in a slightly smaller proportion of cases. It was technically more satisfactory and was positive in nearly half the infants born of heterospecific pregnancies, namely 25 out of 53, in whom the test was applied. This group of 25 infants with a positive indirect antiglobulin test included 14 of the 15 infants with a positive *direct* antiglobulin test and all of the 8 infants who developed hemolytic disease.

These findings may be contrasted with the rather more clear-cut results obtained by Gunson (53) who also found the indirect antiglobulin test positive in nearly all affected infants (39 out of 42) during the first 24 hours of life, but consistently negative in unaffected babies. Zuelzer and Kaplan (50) reported the test as positive in only 1 out of 28 cord blood specimens from heterospecific pregnancies.

As mentioned, 15 out of 25 infants (60%) who exhibited a positive indirect test also showed the antiglobulin test positive in its direct form. The proportion of infants in our series who showed a positive direct test in heterospecific pregnancy is larger than that encountered by other authors. This is presumably due in part to the fact that we routinely tested cord bloods. If blood specimens are taken later the reaction is liable to prove negative soon after birth. However, it is probably at least as important that we used a native antiglobulin serum in serial dilutions, as well as the commercial type. We obtained optimal results at a dilution of native serum to 1:100. Commercial antiglobulin serum is equivalent to a greater dilution of the native material (probably more nearly

1:500) and is most suited for the detection of Rh antibodies and other "warm" agglutinins; for the optimal detection of ABO antibodies more concentrated antiglobulin serum appears to be more suitable. In future it might be advisable to apply this dilution technique also to the indirect antiglobulin test in the investigation of ABO incompatibility.

### Summary

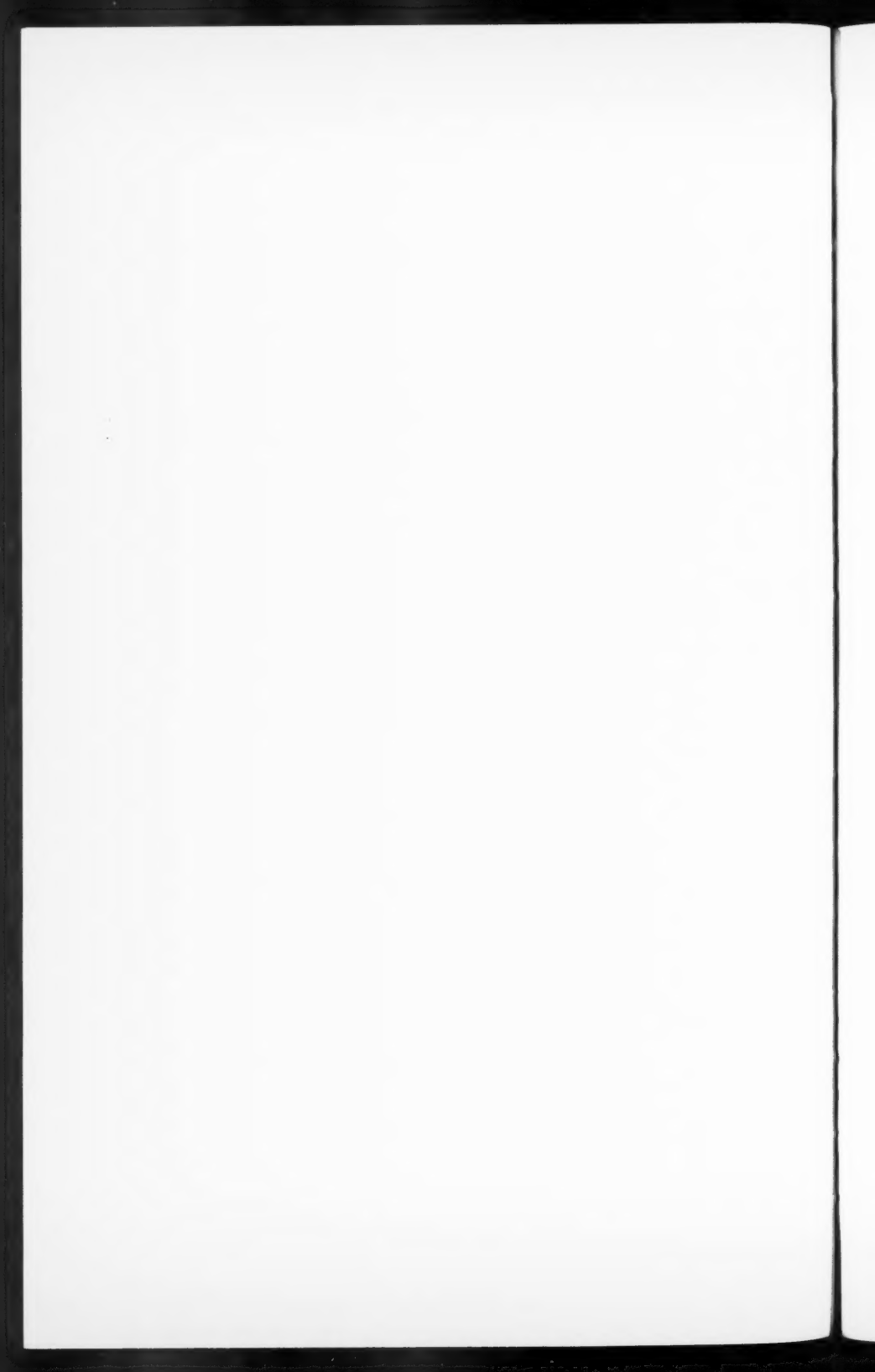
In homospecific O-O pregnancy anti-A and anti-B agglutinins were found to reach the cord blood in the great majority of cases, both as demonstrated in saline at room temperature and in albumin at 37°. The level of saline-agglutinin at room temperature in the cord blood was, however, considerably more depressed in comparison to the maternal titre than that of the agglutinin demonstrated in albumin at 37°. In more than half the cases the titre of agglutinin in albumin in the cord blood exceeded that of the saline-agglutinin. The direct antiglobulin test was uniformly negative in the cord blood of homospecific infants.

In heterospecific pregnancy incompatible saline-agglutinin was only found in the cord blood of 3 out of 55 infants, and at very low titre. One of the 3 babies concerned had a positive direct antiglobulin test. Agglutinin in albumin was demonstrated in 28 of 46 cord bloods tested, but was present in much lower

titre than in homospecific pregnancy. Five of the 8 heterospecific infants who had agglutinins in albumin to a titre of 8 or more also had a positive direct antiglobulin test. The indirect antiglobulin test was positive in about half the infants in whom it could be performed. Virtually all the infants who had a positive direct antiglobulin test were included among those with a positive indirect test, and they represented about 60 % of that group.

The direct antiglobulin test was positive in 15 cases among the 55 heterospecific infants. This represents a larger proportion than that encountered by other workers and is explained by the fact that cord blood was tested routinely and that serial dilutions of native antiglobulin serum were used in addition to commercially available serum. Optimal results were obtained when the native serum was diluted 1:100.

When the positive direct antiglobulin test in the infant is related to the maternal serological status, it is found to occur most frequently when the mother has all the various forms of immune ABO antibodies (hemolysin, residual incomplete agglutinin and positive indirect antiglobulin test after neutralization). In regard to individual maternal antibodies the closest connection appears to be with a positive maternal indirect antiglobulin test after neutralization, for in the presence of this phenomenon the infant had a positive direct antiglobulin test in about half the cases.



PART III

Clinical and Laboratory Findings  
in Heterospecific Pregnancy, with a Note on  
the Incidence of ABO Hemolytic Disease

*by*

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### PART III

## Clinical and Laboratory Findings in Heterospecific Pregnancy, with a Note on the Incidence of ABO Hemolytic Disease

During recent years a number of studies have been undertaken to compare the hematological findings and serum bilirubin values in infants born of homospecific and heterospecific pregnancy, respectively. In 1949 Bryce and her colleagues (57) in Australia examined the hemoglobin level in a fairly large series of newborn children and found that the mean concentration in skin-prick specimens was significantly lower in babies born of heterospecific pregnancy than in homospecific pregnancy. In 1953 Johnstone (58) compared the serum bilirubin values of cord blood in 1078 homospecific pregnancies with those in 275 heterospecific pregnancies and found that the mean in the heterospecific cases was 1.36 mg per 100 ml and significantly higher than the mean value of 1.24 mg per 100 ml in homospecific pregnancies. This difference was unrelated to the Rh status. The author suggested that the difference was accounted for by a greater proportion of readings over 2 mg of bilirubin per 100 ml in the heterospecific group and that some of these could be due to maternal ABO iso-immunization. In 1955, Rosenfield (49) compared the cord blood hemoglobin value, reticulocyte count and serum bilirubin level in a large series of homospecific and heterospecific pregnancies. He also applied the direct antiglobulin test to these cord specimens and considered it positive if as little as 20 % of

the red cells seemed microscopically agglutinated into aggregates of 3 or more. In this way he obtained a weakly positive direct antiglobulin test in over 11 % of group-incompatible infants but in none of the group-compatible children. The infants with this weakly positive direct antiglobulin test had a significantly low mean hemoglobin value and significantly high mean reticulocyte count and serum bilirubin level compared to other group-incompatible infants. It was also interesting that 38 out of 39 mothers of this particular class of group-incompatible infants with positive direct antiglobulin test belonged to group O. This predominance of group O among the mothers was significant, and considering only the infants born of heterospecific pregnancies in which the mother belonged to group O, the incidence of a positive antiglobulin test was 17 %. Thirty-one out of these 38 infants with a positive direct antiglobulin test also had increased osmotic fragility of their red cells, again suggesting that they were actual cases of mild hemolytic disease due to ABO iso-immunization. However, when the incompatible cases with a negative direct antiglobulin test were compared with compatible infants they still had a higher mean reticulocyte count, though not a significantly different level of hemoglobin or bilirubin, and the difference in the reticulocyte count was again found to be

associated almost entirely with group O mothers. Thus it appeared that the positive antiglobulin test still did not detect all the involved cases. None of the affected infants required replacement transfusion, but three were given simple transfusions for anemia.

The statistically significant differences observed in these large series of cases are evidently difficult to demonstrate when smaller groups of infants are compared. Thus Zuelzer and Kaplan (36) studied infants born of homospecific and heterospecific pregnancies and compared them with respect to the occurrence of early jaundice, serum bilirubin levels in cord blood and on the third day of life, hemoglobin levels, spherocytosis, the incidence of splenomegaly or hepatomegaly, and general clinical behaviour. No appreciable differences were apparent between the two groups except for a slightly higher average of the bilirubin levels of questionable significance in the O-A and O-B heterospecific groups. They studied mostly Negro children in whom early jaundice may be difficult to detect, but they found no cases of icterus praecox in a random series of 131 infants born of heterospecific

pregnancy. They concluded that heterospecific pregnancy per se does not constitute a physiological handicap to the infant by the usual clinical and laboratory standards. However, they did find a reduced survival time of those erythrocytes which were agglutinable by the maternal anti-A or anti-B antibodies in seemingly normal heterospecific secretor infants. This selectively increased destruction of fetal red cells did not appear to be of any consequence.

In view of these somewhat discordant findings we considered it of interest to study the incidence and onset of jaundice and to analyse the laboratory findings among the 55 infants born of heterospecific pregnancies in our prospective series. As previously mentioned, the cord blood of 15 among these 55 babies gave a positive direct antiglobulin test and thus divided off a group of seemingly affected babies as in Rosenfield's series.

## Results

The hemoglobin values, reticulocyte counts and serum bilirubin determinations which were performed on the cord

TABLE 19. *Comparison of mean bilirubin, reticulocyte count and hemoglobin concentration of cord blood of infants from homospecific and heterospecific pregnancies when the mothers are group O.*

	Homospecific		Heterospecific		Mean difference	Probability * less than
	Sample size	Mean	Sample size	Mean		
Bilirubin mg/100 ml	68	1.65	34	1.78	0.13	0.30
Reticulocytes %	53	4.55	24	4.50	0.05	0.85
Hemoglobin gm./100 ml	62	16.44	29	16.29	0.15	0.50

\* Probability 0.05 critical value.

TABLE 20. *Comparison of mean bilirubin, reticulocyte count and hemoglobin concentration of cord blood of infants from heterospecific pregnancies divided into two groups on the basis of the direct antiglobulin test.*

	Direct AHG test positive		Direct AHG test negative		Mean difference	Probability* less than
	Sample size	Mean	Sample size	Mean		
Bilirubin mg/100 ml	13	2.00	21	1.64	0.36	0.25
Reticulocyte %	8	5.11	16	4.20	0.91	0.70
Hemoglobin gm./100 ml	11	16.40	18	16.22	0.18	0.30

\* Probability 0.05 critical value.

blood specimens of our homospecific and heterospecific cases are analysed in Table 19. This shows that the serum bilirubin level is indeed somewhat higher and the hemoglobin concentration somewhat lower in the heterospecific group, but the differences are not statistically significant. In Table 20 the infants born of heterospecific pregnancy are divided into two groups according to the result of the direct antiglobulin test. Again the differences in regard to bilirubin level, reticulocyte count and hemoglobin concentration are not statistically significant. Although a statistical analysis of these laboratory data did not show any marked difference between the various groups it was evident that several infants suffered from "icterus praecox" with early jaundice and some abnormal laboratory findings. In order to determine the incidence of "hemolytic disease" among these babies it was necessary to establish some definite criteria of abnormality. It was therefore agreed that a diagnosis of hemolytic disease should be made under the following conditions:

Demonstration of ABO incompatibility between mother and child with exclusion of other sensitization.

2. Positive direct antiglobulin test in the infant and/or positive indirect antiglobulin test of the infant's serum against adult cells of the infant's own group.

3. Either or both of the following: (a) Jaundice in the first 36 hours of life with a serum bilirubin level of at least 10 mg % on the first day of life or of 15 mg % during the first week. (b) Abnormally low hemoglobin concentration (as defined by Mollison (59)) during the neonatal period.

It is realized that there are other useful diagnostic indications of the disease such as spherocytosis or increased osmotic fragility of red cells or reticulocytosis above 7 % during the first two days (41) in the infant; the presence of "immune" ABO antibodies or of a greatly raised titre of saline anti-A or anti-B agglutinins in the mother; or the demonstration of incompatible "immune" or, more rarely, saline-agglutinins in the infant's serum, but these are not included among the essential criteria. Again, a reduced survival time of transfused erythrocytes of a homologous group in the infant is probably the most sensitive index of hemolysis but was excluded from the criteria as it is not a

TABLE 21. *Clinical and laboratory findings in 7 infants from heterospecific pregnancy with positive direct antiglobulin test with hemolytic disease. (BT = simple blood transfusion. NT = not tested. RT = replacement transfusion.)*

Case no.	Parity	Gestational age (weeks)	Birth weight lbs. oz.	Onset of jaundice - hours after birth	Hemoglobin gm./100 ml	Bilirubin mg/100 ml	Therapy
41	3	38	5.15	24	Cord 16.4	Cord 2.6 43 hrs 10.7 67 hrs 12.6 93 hrs 18.2 7 days 16.0	Nil
43	1	42	6.15	27	Cord 15.4 30 hrs-15.9 72 hrs-16.6 7 days 12.4 14 days 9.2	Cord 2.2 30 hrs 8.0 54 hrs 8.0 72 hrs 8.9	B.T. at 3 & 7 weeks
44	8	36	8.11	24	72 hrs-20.8 6 days 16.5	36 hrs 9.0 72 hrs 16.0 90 hrs 15.0	Nil
48	1	35	6.4	24	Cord 17.5 11 hrs-18.5 86 hrs-18.7	Cord 1.8 11 hrs-3.2 54 hrs-16.2 61 hrs-18.2 78 hrs-22.4 86 hrs-22.9 108 hrs-14.0	R.T. at 86 hrs
50	3	45	8.8	22	Cord 15.4	Cord 3.3 2 hrs-4.3 26 hrs-9.7 75 hrs-15.2 99 hrs-12.0	Nil
53	2	41	7.12	12	41 hrs-20.8 84 hrs-22.0	Cord-2.1 41 hrs-11.3 60 hrs-19.2 84 hrs-15.8	Nil
54	2	41	5.4	24	Cord 16.5	Cord 2.3 27 hrs-8.0 54 hrs-10.4 75 hrs-16.3	Nil

very practical method of diagnosis and possibly too sensitive to indicate "disease".

When the heterospecific infants in our series were analysed according to these criteria, it became evident that definite though mild disease had occurred in 7 of the 15 cases who had a positive direct antiglobulin test. Details of the 7 affected infants are given in Table 21. Among the

10 cases with a negative direct but positive indirect antiglobulin test one definite case (case No. 36)<sup>1</sup> occurred (Table 22). In the remaining 30 cases with both these tests negative there was no clinical or laboratory evidence of disease. Treatment for the entire group was expectant except for a replacement transfusion in one patient

<sup>1</sup> See Table 15.

TABLE 22. Findings in 10 infants of heterospecific pregnancy with negative direct AHG test and positive indirect AHG test. Case no. 36 had definite clinical disease. (NT = Not tested.)

Case no.	Parity	Gestational age (weeks)	Birth weight lbs. oz.	Onset of jaundice -hours	Hemoglobin gm./100 ml	Bilirubin mg/100 ml	Sphero-cytosis	Reticulo-cytosis%	Therapy
9A	7	40	4.8	72	Cord 17.5 72 hrs 18.5	Cord 1.8 72 hrs 11.7	—	Cord 3.1	Nil
23	2	43	7.12	96	Cord 15.5 4 days 6.0	Cord 1.4	—	Cord 6.0	Nil
24	1	37	5.13	60	Cord 14.9	Cord 0.4	—	Cord 4.6	Nil
27	1	39	7.5	none	Cord 16.2	Cord 1.2	—	Cord 3.1	Nil
33	7	40	8.1	none	36 hrs-16.5	36 hrs-5.3	—	N.T.	Nil
34	2	40	7.11	72	Cord 15.1	Cord 2.6 72 hrs 9.9 6 days 12.8	Increased fragility	N.T.	Nil
35	8	40	4.15	none	Cord 15.65	Cord 0.8 56 hrs 0.8	—	Cord 5.9	Nil
36	7	42	7.12	18	Cord 14.2 4 days 19.0	Cord 1.7 4 days 15.7	Sphero in cord	Cord 6.7	Nil
38	2	40	8.4	none	Cord 16.6	Cord 1.1 5 days 1.7	—	Cord 4.8	Nil
39	1	42	9.2	72	Cord 17.7 5 days 17.1	Cord 2.8 5 days 11.2	—	Cord 3.1	Nil

and two simple transfusions for anemia in another patient. Among the homospecific pregnancies there were no cases of hemolytic disease.

Finally, those infants who had a positive direct antiglobulin test and fulfilled the above criteria of hemolytic disease may be compared with the other groups. Table 23 shows that if they are compared with those infants who also have a positive direct antiglobulin test but do not fulfil our third criteria of disease, there is a significant difference in the arithmetic means of the cord serum bilirubin levels of the two groups. This is not altogether surprising as the serum bilirubin level itself will partly determine the presence of disease according to our criteria; it is, however, of interest in showing that the

bilirubin level of the cord blood is a useful index of developing hemolytic disease in these infants. While a bilirubin level of the cord serum above 3 mg % is highly suspicious of hemolytic disease (15), Tables 21 and 22 show that even cord bilirubin values of 1.7 mg % may be followed by disease.

There were three firstborn infants among the eight definite cases of hemolytic disease. Six of these babies were born within two weeks of term and though the remaining two were calculated to have been born at 35 and 36 weeks gestation, they weighed 6 lbs. 4 oz. and 8 lbs. 11 oz., respectively, and did not appear to be immature.

With regard to "icterus praecox", 7 of the 55 infants born of heterospecific

TABLE 23. Comparison of mean serum bilirubin level of cord blood of infants from heterospecific pregnancies with positive direct antiglobulin test and clinical disease and of infants from heterospecific pregnancies with positive direct antiglobulin test and no clinical disease. The mothers were all group O.

Heterospecific (direct AHG test positive)					
With disease		Without disease		Mean difference	Probability* less than
Sample size	Mean Bilirubin mg./100 ml	Sample size	Mean Bilirubin mg./100 ml		
6	2.38	6	1.23	1.15	0.005

\* Probability - 0.05 critical value.

pregnancy in our series were found to be jaundiced at 24 hours after birth or earlier. These 7 babies all had clinical disease according to our criteria. Six of them had a positive and one had a negative direct antiglobulin test; in all 7 the serum gave a positive indirect antiglobulin test against adult cells of their own group (Case Nos. 36, 41, 44, 48, 50, 53 and 54).<sup>1</sup> In 69 homospecific pregnancies, jaundice occurring at 24 hours or less was only observed in one infant (Case No. 39).<sup>2</sup> This baby also still had a serum bilirubin level of 12.6 mg% seven days after birth. The cause of the early and marked jaundice in this case was not established.

When the maternal serological findings are listed for the infants affected by hemolytic disease, no consistent pattern emerges (Table 24). The titre of maternal saline-agglutinin exceeded 1000 in the prenatal period in 3 out of the 8 definite cases. The albumin titre at 37°C showed a two tube excess over the saline titre in only 2 out of 8 cases. All maternal sera contained hemolysin against adult cells of the infant's group before birth. The indirect antiglobulin test of the maternal sera

against adult cells of the infant's group was positive after neutralization of saline-agglutinins in 5 of the 8 mothers, and incomplete agglutinin in albumin was present after such neutralization in 4 of the 8. Fig. 4 shows the serological findings as related to the eventual occurrence of hemolytic disease in 26 infants whose cord blood showed a positive antiglobulin test in either form. This reveals that 7 out of 8 infants with clinical disease belonged to the group of 15 infants who have a

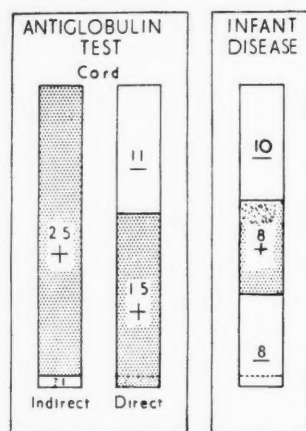


Fig. 4. The relationship between the results of Indirect and Direct antiglobulin tests on cord blood and the eventual occurrence of hemolytic disease in 26 infants in whom the test was positive in either form.

<sup>1</sup> See Part 2 Table 15.

<sup>2</sup> See Part 2 Table 13.

TABLE 24. Prenatal maternal serological findings in 8 cases of ABO hemolytic disease among infants born of heterospecific pregnancies. In case no. 36 the direct antiglobulin test was negative; it was positive in the other 7 cases.

Case no.	Infant's group	Prenatal maternal antibody findings				
		Anti-A or anti-B titre		Hemolysin	After neutralization	
		Saline agglutinin	Agglutinin in albumin		Indirect AHG test	Incomplete agglutinin
36	B	20-320	20-80	8	+	24
41	A	1280-2560	2560	4	+	48-192
43	A	160-80	40	2	-	Nil
44	A	160-320	320	16	-	Nil
48	A	10,240-1280	5120-1280	8-4	+	320
50	A	1280-2560	5120-10,240	8-16	+	Nil
53	B	640	640	4	-	Nil
54	B	640	5120	8	+	256

direct antiglobulin test. Fourteen of these (one not tested) in turn are all among the 25 infants with a positive indirect antiglobulin test of their serum against adult cells of their own group. One infant with definite disease had a negative direct but positive indirect antiglobulin test.

#### Incidence of Hemolytic Disease

The incidence of hemolytic disease due to ABO iso-immunization has been estimated variably by different authors. Halbrecht (29) considered that icterus praecox occurred once in every 25 to 30 heterospecific pregnancies, which is proportionately similar to the incidence of erythroblastosis fetalis in Rh incompatibility. This would mean that disease due to ABO iso-immunization occurs once in every 100-150 pregnancies in general. Similarly Hsia and Gellis (30) examining all infants in a hospital who were jaundiced within the first 36 hours found erythroblastosis due to ABO iso-immunization in 11 out of 150 and later in 14 out of 2624 infants, which represents an incidence of 0.7%. Turnan and his colleagues (60) found an

incidence of 0.8% among 2672 infants born in two hospitals in Pennsylvania. Leikin *et al.* (61) noted 12 cases of ABO hemolytic disease in 230 ABO incompatible infants born of 1125 unselected pregnancies. The incidence of the disease was 1.15% for the total series of pregnancies, 5.6% for the ABO incompatible group and 7.0% when the mother belonged to group O and the infant to A or B. Valentine (62) found 14 cases of ABO hemolytic disease among 1000 unselected births and calculated the incidence of disease as one in seventy-one births and as 7% in ABO heterospecific pregnancies. On the other hand, according to comprehensive statistics the frequency of ery-

TABLE 25. The expected and observed incidence of group O, A and B in 155 offspring of group O mothers mated with fathers of unknown ABO group.

Group of offspring	Observed	Expected
O	100	102
A	41	44
B	14	9
Total	155	155

thrombocytosis due to ABO incompatibility in the Japanese has been calculated as only approximately 0.48 % of all births (63).

Our own series is a relatively small one, but each infant has been fairly fully investigated for the possible presence of the disease. Table 25 shows that the distribution of group O, A and B offspring among the infants we examined is not significantly different from that which would be expected when a random series of 154 group O mothers are mated with fathers of unknown ABO group in the general population, in accordance with the prevalent genotype frequencies.

In random matings between group O mothers and fathers of unknown blood group the frequency of different combinations can be determined from a consideration of genotype frequencies. It is likely that the following percentage of combinations will result, expressed as percentage of all matings (26):

Mother	Father	%
O	A <sub>1</sub> A <sub>1</sub>	1.90
	A <sub>1</sub> O	12.02
	A <sub>1</sub> A <sub>2</sub>	1.26
	A <sub>2</sub> A <sub>2</sub>	.21
	A <sub>2</sub> O	4.01
	BB	.16
	BO	3.52
	A <sub>1</sub> B	1.11
	A <sub>2</sub> B	.37
	OO	19.00
		<hr/> 43.56

As eight definite cases of hemolytic disease due to ABO incompatibility resulted from the 154 matings in our series, the incidence of the disease was 5.1 % and as this type of mating comprises 43.6 % of all matings the incidence in all matings regardless of parental blood group

may be calculated as 2.2 %. Thus, with careful investigation, mild cases of the condition, as defined by our criteria appear to occur more often than has been suggested previously.

Levine (26) has shown from a review of recorded cases that in 98 % of instances of ABO hemolytic disease the mother belongs to group O and the infant to group A<sub>1</sub> or B, or in other words the father may have the genotypes A<sub>1</sub>O, A<sub>1</sub>A<sub>1</sub>, A<sub>1</sub>A<sub>2</sub>, A<sub>1</sub>B, A<sub>2</sub>B, BO, or BB. The frequency of these matings is only 20.35 %, and therefore the incidence of ABO disease among the offspring of group O mothers and such "dangerous fathers" in analogy to our own series can be calculated to be as high as 11.1 %, or one in nine such matings. The incidence of ABO disease in the offspring of the different types of matings is shown in Figure 5. Finally, it should be mentioned that the incidence would probably be found to be still higher if the most sensitive tests of hemolysis were applied to the infant, such as red cell survival studies (36).

### Discussion

Other workers have shown statistically significant differences between means for cord blood bilirubin level, hemoglobin concentration and reticulocyte counts in homospecific and heterospecific pregnancies. The present study shows only slight differences in mean cord blood values for serum bilirubin and hemoglobin concentration in the expected direction. The differences between the means are not of statistical significance, probably because of the small size of the series. However, by defining instances of hemolytic disease according to serological, biochemical and

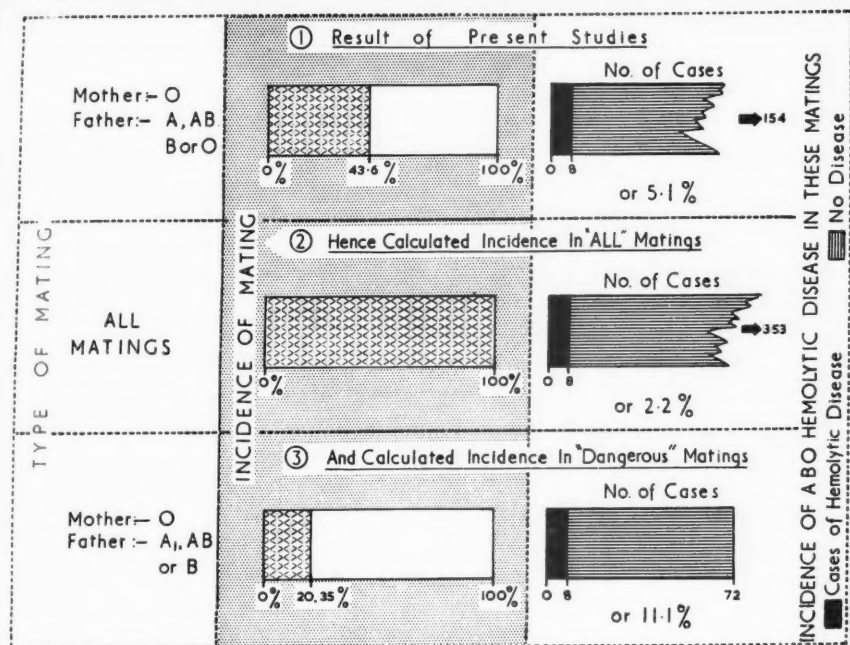


Fig. 5. Incidence of Hemolytic Disease in Offspring of Different Types of Mating.

hematological criteria one can split off a small group of cases which is significantly different from the rest, at least in respect of serum bilirubin level. Johnstone (58) also noted that the difference between the mean cord serum bilirubin values in homospecific and heterospecific pregnancy was accounted for by a relatively small number of infants with high readings in the ABO heterospecific group. He suspected that some of these were possibly due to mother-child ABO iso-immunization, but among 3070 consecutive deliveries he found no instances of erythroblastosis due to maternal ABO iso-immunization; this appears to have been due largely to the fact that the diagnosis was not made in the absence of a positive direct antiglobulin test and that the latter was

not obtained in ABO cases by the technique used.

Our findings are essentially similar to those of Rosenfield (49). By his technique he demonstrated a positive antiglobulin test in 17% of infants born of heterospecific pregnancies in which the mother belonged to group O, and he showed that this group of infants was abnormal in having a significantly low mean hemoglobin concentration as well as a high mean bilirubin level and reticulocyte count. He realized, however, that some of the infants of heterospecific pregnancies whose blood gave a negative direct antiglobulin test also showed abnormalities as indicated by a significantly high mean reticulocyte count compared to compatible infants. Using the titration technique we de-

monstrated a positive antiglobulin test in 27 % of the offspring from heterospecific pregnancies with group O mothers and thus we cast our net somewhat wider. The group of infants with a positive direct antiglobulin test in our series was shown to include 7 out of the 8 cases of hemolytic disease, but the whole series was not large enough to obtain a significant difference in hematological and biochemical data as compared to the rest of the group. Only the cases of actual disease showed a significant difference in serum bilirubin levels.

Zuelzer and Kaplan (36) in their series similar to our own, found no appreciable differences between infants born as the result of homospecific and heterospecific pregnancies except for a slightly higher average of the bilirubin levels of questionable significance in the heterospecific group. This difference was not regarded as significant for the size of their sample, but it agreed with the figure obtained by Rosenfield (49) in his much larger series. Our results are at variance with those of Zuelzer and Kaplan in that they found icterus praecox in none of 131 infants born of heterospecific pregnancies, whereas we detected jaundice within 24 hours in 7 out of 55 such infants and were able to show laboratory evidence of hemolytic disease in another 1. Therefore we cannot agree with their conclusion that heterospecific pregnancy per se does not constitute a physiological handicap to the infant by the usual clinical, hematological and biochemical standards.

### Summary

With the use of a titration method applied to native antiglobulin serum, 15 out

of 55 infants born of heterospecific pregnancy were found to have cord blood giving a positive direct antiglobulin test. Neither the whole group of heterospecific infants nor the sub-group with a positive direct antiglobulin test differed significantly from homospecific infants in regard to the mean hemoglobin concentration, serum bilirubin level or mean reticulocyte count. However, by laying down certain serological, clinical, biochemical and hematological criteria of hemolytic disease, it was possible to separate a group of 8 infants with disease in the heterospecific group who differed significantly from homospecific infants and even from the other heterospecific infants with a positive direct antiglobulin test in regard to their mean cord serum bilirubin level. Details of the clinical status, hemoglobin concentration and serum bilirubin levels and other hematological data in regard to these affected infants are given. The serological pattern in their mothers is also tabulated.

The occurrence of 8 cases of hemolytic disease in the offspring of 154 random group O mothers mated with fathers of unknown ABO group represents a disease incidence of 5.1 %. On the basis of this incidence in heterospecific pregnancy it is calculated that the disease probably occurs in about 2.2 % of all pregnancies and in 11.1 % of "dangerous matings" when the mother belongs to group O and the father has an A<sub>1</sub> or B agglutino-gen or both.

It is agreed that the disease is usually mild; among 55 heterospecific infants only one required a replacement transfusion, and one had to have two simple transfusions for late anemia.

PART IV

Hemolytic Disease of the Newborn Due to  
ABO Iso-Immunization  
Clinical and Laboratory Findings in 37 Cases, with Special  
Reference to Serological Studies

*by*

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## PART IV

### Hemolytic Disease of the Newborn Due to ABO Iso-Immunization

Within the past decade hemolytic disease due to ABO incompatibility has become recognized as a common condition in the newborn, usually manifested by early jaundice and often by anemia. The earlier reports of isolated cases have been succeeded by studies of larger series, and much work has been done on the laboratory diagnosis of the disease. It has become apparent that in well over 90 % of cases the mother belongs to group O (25) (26) (15) (27), and the infant's group is usually A<sub>1</sub> or, less commonly, B (64). With regard to hematological findings Grumbach and Gasser (23) first pointed out in 1948 that affected infants exhibit spherocytosis and increased osmotic fragility of the red cells. This was demonstrated independently in four affected infants by one of us (28) with another group of investigators, and in one later series of 11 babies with hemolytic disease due to anti-A all were reported as having spherocytosis and 10 as showing increased red cell fragility (41). The frequent marked reticulocytosis has also been emphasized (64) (65) and is usually somewhat more impressive than the number of nucleated red cells.

With regard to the serological findings, Wiener and his collaborators first demonstrated that the maternal immune (univalent) anti-A and anti-B agglutinins

can traverse the placenta and are liable to cause disease in the infant, whereas high maternal titres of saline-agglutinins (bivalent) are not necessarily correlated with such disease (2). The importance of maternal "immune" ABO antibodies was confirmed in a series of 45 patients reported from Holland in 1950 in whom not only the maternal agglutinin titres were estimated but also the indirect antiglobulin and blocking tests were performed on the mother's serum after partial absorption with A or B substance (66). Other workers have emphasized that the group O mothers of affected group A infants constantly exhibit anti-A hemolysins (41) (67). Unfortunately this is of limited diagnostic value as it is a common finding in the general population and is almost universal in heterospecific pregnancy (cf. Part I of this paper). Crawford and his colleagues (41) have made the test more selective by checking the maternal serum for hemolysins against the relatively non-reactive red cells of the infant rather than against adult red cells as usual. However, using this modified technique, Zuelzer and Kaplan (64) found the test positive in only 13 out of 18 mothers of affected infants. Tests for other maternal "immune" antibodies have the same defect of being inconstantly positive when the infants are affected by the disease. Thus the indirect

TABLE 26. *Maternal and infant serological findings together with other pertinent data in 36 cases. PP = post partum, R = Rh.*

Maternal Serology											
Case no.	Prev. preg.	Maternal group and type	Time of sample	Hemo-lysin titre	Antibody titre			After neutralization		Infant group and type	Time of sample
					saline at room temp.	albu-min at 37°		Indirect AHG test	Incomplete agglutinin		
1	3	O, D	8 days PP	4	10,240	81,920	NT	NT	B, CDe/CDe	42 hrs	
2	3	O, CDe/cde	1 day PP	4	2560	NT	+	40	A, D	38 hrs	
3	2	O, D	13 days PP	4	10,240	10,240	+	—	A, D	32 hrs	
4	0	O, CDe/CDe	1 day PP	4	640	2560	+	—	A, CDe/CDe	5 hrs	
5	0	O, D	1 day PP	4	80	NT	+	16	A, D	Cord	
6A	1	O, D	8 days PP	4	2560	640	+	20	A, D	8 days	
B									A, D	8 days	
7	2	O, D	2 days PP	4	1280	10,240	—	—	B, D	40 hrs	
8	2	O, D	9 days PP	4	5120	5120	+	—	A, D	24 hrs	
9	0	O, CDe/cde	5 days PP	4	320	NT	+	20	A, cDe/cde	21 hrs	
10	2	O, D	1 day PP	8	640	NT	+	—	A, D	16 hrs	
11	5	O, cde/cde	1 day PP	4	2560	NT	+	20	A, cde/cde	36 hrs	
12	3	O, D	4 days PP	8	320	NT	+	40	B, D	12 hrs	
13	2	O, D	1 day PP	4	5120	NT	—	—	A, D	23 hrs	
14	3	O, D	1 day PP	4	80	640	+	16	A, CDe/cde	22 hrs	
15	2	O, D	1 day PP	2	320	320	+	20	A, D	25 hrs	
16	2	O, D	1 day PP	4	2560	640	+	48	A, D	56 hrs	
17	0	O, D	1 day PP	4	1280	1280	—	48	A, CDe/cDE	9 hrs	
18	1	O, D	1 day PP	16	10,240	10,240	+	160	A, D	7 hrs	
19	0	O, D	8 days PP	16	640	320	+	—	A, D	36 hrs	
20	0	O, CDe/cde	1 day PP	4	5120	5120	+	40	A, CDe/cde	25 hrs	
21	3	O, CDe/CDe	2 days PP	8	10,240	10,240	+	512	A, CDe/cde	35 hrs	
22	0	O, CDe/cde	1 day PP	4	5120	320	—	—	A, CDe/cde	4 hrs	
23	2	O, CDe/cde	1 day PP	—	640	320	+	—	A, CDe/cde	21 hrs	
24	2	O, D	7 days PP	NT	2560	2560	+	192	A, D	43 hrs	
25	0	O, D	3 days PP	2	80	40	—	—	A, dd	30 hrs	
26	7	O, D	4 days PP	16	320	320	—	—	A, D	15 hrs	
27	1	O, D	3 days PP	8	640	640	—	—	B, D	48 hrs	
28	4	O, cDE/cde	3 days PP	8	640	640	+	48	B, D	cord	
29	0	O, cde/cde	1 day PP	8	1280	1280	+	320	A, dd	11 hrs	
30	1	O, D	2 days PP	4	80	NT	+	16	B, D	48 hrs	
31	1	O, D	1 day PP	4	640	640	—	—	A, D	96 hrs	
32	2	O, D	1 day PP	4	2560	10,240	+	—	A, cde/cde	26 hrs	
33	1	O, cde/cde	3 days PP	2	2560	320	—	—	A, D	79 hrs	
34	1	O, CDe/cde	1 day PP	4	160	320	+	—	B, CDe/cde	26 hrs	
35	1	O, CDe/CDe	1 day PP	4	2560	2560	+	160	A, CDe/cDe	12 hrs	
36	3	O, CDe/cDE	1 day PP	8	1280	5120	+	32	A, cDE/cde	13 hrs	

antiglobulin test against cells of the baby's group was positive in maternal serum after neutralization of saline-agglutinins in 3 out of 9 cases in one series (67) and in 22 out of 35 in another (64).

As one of us has previously pointed out

(31) the demonstration of ABO incompatibility in the absence of any other and the presence of anti-A or anti-B saline-agglutinins in high titre or of immune ABO antibodies in the maternal serum do not, in any case, represent conclusive

specific pregnancies. AHG = antihuman globulin, B.T. = simple blood transfusion, NT = not tested, B.T. = simple blood transfusion.

														Infant Hematology				
Direct AHG test				In-direct AHG test	Onset of jaun- dice	Maximal Bilirubin		Minimal Hemoglobin		Reticu- culo- cytes %	Nucle- ated RBC/100	Sphero- cytes	Therapy					
Native	AHG	Serum	mg/ 100ml			Hour	gm./ 100ml	Hour										
1/50	1/100	1/200	1/500															
NT	NT	NT	NT	+	NT	14 hrs	42	10.6	42	12.2	120	8	0	1+	R.T. 48 hrs			
															B.T. 3 days			
NT	NT	NT	NT	+	NT	24 hrs	38	18.2	38	16.7	38	0	0	1+	R.T. 45 hrs			
NT	NT	NT	NT	+	NT	24 hrs	40	18.2	32	16.0	32	NT	0	4+	R.T. 40 hrs			
NT	NT	NT	NT	+	-	3 hrs	5	7.7	5	14.8	5	28	75	NT	R.T. 10 hrs			
NT	NT	NT	NT	+	-	24 hrs	58	19.0	36	17.0	36	12	NT	NT	None			
NT	NT	NT	NT	+	NT	Birth		NT		NT		NT	NT	NT	None			
NT	NT	NT	NT	+	NT	Birth		NT		NT		NT	NT	NT	None			
-	+	+	-	+	NT	36 hrs	57	26	48	11.4	48	9.8	0	NT	R.T. 57 hrs			
NT	NT	NT	NT	+	NT	6 hrs	48	21	24	17.3		NT	NT	NT	R.T. 52 hrs			
-	+	+	+	+	NT	21 hrs	46	14.3	70	16.3	21	7	NT	NT	None			
-	+	NT	+	+	-	12 hrs	16	9.4	16	17.7	16	12.7	10	1+	None			
-	-	-	-	+	NT	24 hrs	60	19.0	36	17.0	36	NT	NT	1+	None			
-	-	NT	NT	+	NT	8 hrs	11	10.8	11	19.2	11	8.4	140	1+	None			
-	-	NT	+	+	+	6 hrs	24	15.9	24	18.5	24	2.8	3	1+	None			
-	-	-	-	+	NT	8 hrs	22	10	22	14.8	24	NT	3	NT	R.T. 12 hrs			
NT	NT	NT	NT	+	-	20 hrs	46	9.7	46	13.4	25	18	8	1+	None			
-	+	+	+	+	NT	32 hrs	32	15.1	32	13.8		NT	NT	NT	None			
NT	+	+	+	+	NT	7 hrs	36	7.4	24	14.3	24	27	4	2+	None			
-	+	+	NT	+	8	7 hrs	31	14.3	96	11.9	7	18	18	NT	None			
-	+	+	NT	+	NT	36 hrs		NT	36	11.0	72	7	NT	NT	None			
-	+	+	NT	+	4	14 hrs	24	15.1	72	17.0		NT	NT	NT	None			
-	+	+	+	+	4	24 hrs	35	13.7	35	14.5	35	14	1	NT	None			
-	+	+	+	+	16	4 hrs	60	32.7	76	12.4		NT	NT	NT	R.T. 60 & 82 l			
															B.T. 5 days			
-	+	+	-	+	2	18 hrs	96	17.6	21	15.6		NT	NT	NT	None			
-	+	+	+	+	8	24 hrs	93	18.2	93	16.0	43	NT	NT	2+	None			
-	+	+	+	+	-	27 hrs	30	8.8	30	15.9	30	NT	NT	1+	2 B.T.			
-	+	+	-	+	4	24 hrs	60	16.0	60	20.8		NT	NT	NT	None			
-	+	+	+	+	4	12 hrs	61	19.2	41	20.8		NT	NT	NT	None			
-	+	+	+	+	128	20 hrs	24	15.0	24	18.8	cord	5.8	65	NT	None			
-	+	+	+	+	8	20 hrs	78	22.4	11	18.5	11	7.6	44	2+	R.T. 86 hrs			
NT	NT	NT	NT	+	NT	12 hrs	80	16.3	48	15.2		NT	NT	NT	None			
-	+	+	+	+	NT	4 hrs		NT	72	12.0	48	NT	12	NT	None			
-	+	+	-	+	2	24 hrs	75	15.2	cord	15.4	cord	7.4	18	4+	None			
-	+	+	-	+	8	24 hrs	79	16.3	79	18.8	79	3.7	0	2+	None			
-	+	+	-	+	16	19 hrs	26	17.8	26	15.9	26	13.7	2	NT	R.T. 25 hrs			
-	+	+	+	+	2	12 hrs	47	17.8	47	14.7	12	8.5	8	NT	R.T. 47 & 68 l			
-	+	+	+	+	8	12 hrs	24	14.7	22	13.6	13	11.9	22	4+	R.T. 22 & 48 l			

serological evidence for the disease. Additional proof should be supplied by showing that the incompatible antibodies are present in the infant, particularly if their action can be demonstrated, at least by a positive direct antiglobulin test.

The incompatible antibody can be most conveniently detected in the infant's serum as an incomplete agglutinin or by means of the indirect antiglobulin technique against adult cells of the infant's group. As mentioned in Part II of this

paper, the latter method was more satisfactory and appeared more sensitive in our hands. Using both these techniques Zuelzer and Kaplan (64) demonstrated free incompatible antibody in 26 out of 29 infants with ABO hemolytic disease; Crawford and his colleagues (41) found the indirect antiglobulin test against adult A cells positive in 8 of 11 affected infants. The remaining babies may, of course, also be presumed to have had incompatible antibody transmitted but represent "serological failures".

It has long been known that the direct antiglobulin test may be negative in regard to cells which are only weakly sensitized by anti-A or anti-B agglutinins (68). Thus it is not surprising that the test has been negative in many infants reported as suffering from ABO hemolytic disease. A negative direct antiglobulin test has even been described as an expected finding in the disease (30), though the test has also been reported as definitely, or at least weakly, positive in a proportion of affected babies by various authors. For instance, in one series 6 of 37 infants had a weakly positive direct antiglobulin test (64), and in another group the test was positive in 32 out of 134 newborn (24%) with ABO disease (15). Various attempts have been made to devise a more sensitive modification of the test, such as a microscopic technique (49) or the two-stage antiglobulin reaction of Richardson Jones which is reported as positive in over 60% of cases (30). One of the simplest and most effective modifications would appear to be the use of potent native absorbed antiglobulin serum in serial dilution (55) (41), and we have found this method quite successful, as reported in previous sections

of this paper. If desired, the incompatible antibody can be eluted from the surface of the red cells which give a positive direct antiglobulin test and may then be identified as anti-A, or anti-B (56).

The most sensitive diagnostic test presently available is the study of the differential survival rate shown by red cells of different groups transfused into the infant, but this is unfortunately impracticable as a routine hospital procedure. It may also be objected that it is such a sensitive test as to show up fully compensated reductions in red cell survival which hardly amount to a "hemolytic disease" (36).

We now present our own experiences in a recent series of 37 cases of hemolytic disease due to ABO incompatibility, with particular reference to the serological findings.

### Case Material and Methods

While the prospective study of pregnant group O women and their offspring, reported in previous sections of this paper, was in progress at The Vancouver General Hospital, the Pediatric Research Laboratory of the Health Centre for Children rendered diagnostic services to any staff or private patient in various local hospitals in whom the diagnosis of ABO hemolytic disease of the newborn was suspected. In this way, a fairly large number of instances of the disease came to the notice of the present authors. However, it should be noted that the writers were not responsible for the management of most of these patients and had only limited facilities for investigation. The cases which will now be analyzed had

fairly full serological investigation and fulfilled the diagnostic criteria for hemolytic disease laid down in Part III of this paper, viz:

1. Demonstration of ABO incompatibility between mother and child, with exclusion of any other sensitization.

2. Positive direct antiglobulin test in the infant and/or positive indirect antiglobulin test of the infant's serum against adult cells of the infants' own group.

3. Either or both of the following:

- (a.) Jaundice in the first 36 hours of life with a serum bilirubin level of at least 10 mg % on the first day of life or of 15 mg % during the first week.

- (b.) Abnormally low hemoglobin concentration (as defined by Mollison (59) during the neonatal period.

37 such affected infants were available for analysis. Among them were two surviving triplets, hence 36 mothers are comprised in the study. The mothers all belonged to group O; 33 were Rh positive, and 3 were Rh negative without Rh antibodies. 30 infants had blood group A and 7 had group B; the A subgroup was not determined.

The laboratory methods used were those described in the introduction to this paper. Titrations of maternal anti-A or anti-B agglutinins were performed in saline at room temperature and in albumin at 37°C. The maternal sera were also tested and titrated for hemolysin against adult A and B cells and frequently against the infant's cells. After complete neutralization of saline-agglutinins with A and B group specific substance, indirect antiglobulin tests were performed and agglutination titration in albumin was repeated. The infants were examined clinically,

various hematological tests were performed and the serum bilirubin level was estimated. Serological studies in the infants included serum agglutinin titration in albumin at 37°C and indirect antiglobulin test against adult cells of their own group, as well as direct antiglobulin test on the infant's red cells, using both commercial antiglobulin serum and various dilutions of a native absorbed antiglobulin serum.

## Results

The main data concerning these cases are listed in Table 26.

### *I. Clinical, hematological and biochemical findings in the infants*

In regard to the pregnancy history, it is notable that 9 of these 37 babies were first-born. In the remaining 28 infants (including the two surviving triplets), a history suggestive of hemolytic disease in previous pregnancies was obtained in 9 instances and an exchange transfusion had been administered in one of these (Case No. 28). Many of these histories were not sufficiently reliable in retrospect to warrant further detailed analysis.

The infants had all been born within two weeks of term, and 35 of the 37 had a normal delivery and birth weight. The exceptions are the two surviving triplets who were born in another city at 38 weeks' gestation after labour had been induced for mild toxemia; they weighed 4 lbs. 6 oz. and 5 lbs. 11 oz., respectively (Case Nos. 6A and 6B), and were brought to our notice after the third had died of kernicterus.

The babies had generally appeared well at birth. Most of them attracted their physician's attention by the early develop-

TABLE 27. *Hematological findings in infants with ABO hemolytic disease, preceding transfusions.*

Time of test	No. of infants tested		No. of tests performed		Range	Mean value
<i>1. Hemoglobin concentration (gm./100 ml)</i>						
1st day	20		28		12.4-21.0	16.3
2nd day	20		22		11.0-20.8	15.2
3rd-7th days	15		24		11.9-22.0	15.8
<i>2. Reticulocyte count (%)</i>						
1st day	13		18		2.8-28	10.6
2nd day	6		6		9.8-18	13.7
3rd-7th days	4		4		3.7- 8	6.2
<i>3. Nucleated red cell count (per 100 W.B.C.)</i>						
1st day	13		18		1-140	29.5
2nd day	5		5		0- 12	5.8
3rd-7th days	3		3		0- 9	3.7
<i>4. Spherocytes</i>						
Grade of spherocytosis	0	+ 1	+ 2	+ 3	+ 4	Total infants tested
No. of infants tested	0	8	4	0	3	
						15

ment of jaundice. Usually this was an unexpected phenomenon and cord blood specimens were only available for examination in 10 babies. In 33 of the 37 infants jaundice was noted within 24 hours of birth; in the remaining 4 cases it became apparent at 25-36 hours. On only one occasion was it claimed that jaundice had been present at birth; this was in the case of the triplets who were born in another city; their serum bilirubin levels were not determined, and blood specimens for serological tests were only obtained 8 days after birth from the two surviving infants. We have no cases of hemolytic disease presenting as anemia without jaundice in the series, though we have seen isolated cases which are not included here, and well-documented instances have been reported by others (41) (64). However, pallor was noted in addition to jaundice in

5 cases in our series. On the whole, it appeared less common than in cases of hemolytic disease due to Rh incompatibility. Similarly, distinct hepatomegaly was unusual and a palpable spleen was only noted on the first day in 10 of the 37 infants. No abnormal signs were observed in the nervous system of any of these patients.

The hematological investigations are listed in Table 27. In 10 of the 35 infants in whom it was estimated, the *hemoglobin concentration* was below the level of 14.5 gm % which is often taken as the lower limit of normal in venous blood during the first 2 or 3 days of life (69) (59). The mean values of hemoglobin concentration in these babies are also seen to be low compared to the normal. Yet early gross anemia (below 11 gm %) was not encountered in this series. The *reticulocyte count* again

showed a wide range. In 12 of the 20 infants in whom they were performed the counts exceeded the upper limit of normal which has been defined as 7% during the first two days and 5% from the third day onwards (41), and their mean values remained above that limit beyond the second day. The nucleated red cell count was above 10 per 100 W.B.C. in 7 of the 17 babies in whom it was examined, and the mean value of 29.5 on the first day is well above the upper limit of normal, though the means on subsequent days suggest a rapid fall to more normal levels. The blood films of 15 infants were examined for red cell morphology; marked spherocytosis (+2 to +4) was found in 7 of these. Red cell fragility was tested in only six infants and was found to be increased in only two, who both exhibited spherocytosis.

In regard to these hematological findings, it will be seen that the result of repeated tests in the same patients have been included in the analysis, and it may be argued that this might affect the mean values as the more seriously affected babies are more likely to have repeated tests. On the other hand, only findings preceding

transfusions have been analyzed, and this would tend to bias the analysis in the reverse direction.

The serum bilirubin levels are analyzed in Table 28. It will be seen that the range was wide but some high levels were reached and the means and maximal values would presumably have been still higher if replacement transfusions had not been performed in 12 of the 37 infants. Excluding post-transfusion figures the mean values rose to 15.7 mgm% on the third day, then fell. In 4 cases the serum bilirubin was allowed to rise above 20 mgm%, reaching values of 21, 22.9, 26 and 32.7 mg% before exchange transfusion was performed, yet no obvious ill-effect was noted.

The clinical course in these infants was generally satisfactory and none developed signs of kernicterus. As mentioned, altogether 12 of the 37 babies received replacement transfusions. In 3 cases this was repeated and two of the patients subsequently had a simple transfusion. One further infant received only two simple transfusions. The jaundice generally subsided about the beginning of the second week, but in at least one case marked icterus persisted beyond this period. The anemia occasionally showed slow deterioration; thus in one full term infant the hemoglobin concentration fell to 8.9 gm% at 4 weeks.

TABEL 28. Serum bilirubin levels in infants with ABO hemolytic disease (excluding premature babies and specimens taken after transfusion).

Time	No. of		Range	Mean value
	infants	specimens taken	mg/100 ml	mg/100 ml
1st day	25	32	1.6-16.6	7.6
2nd day	29	35	6.0-20.8	13.0
3rd day	19	23	6.6-32.7	15.7
4th day	12	13	4.2-22.9	14.7
5th-7th days	10	10	2.7-16.3	10.7

## II. Maternal serological findings

1. Saline-Agglutinins. The ranges in saline-agglutinin titre in the early post-partum period were again broad, as noted in our prospective studies. The saline anti-A titre in the first week after delivery extended from 80 to 10,240 with a median

titre of 1280 in 30 mothers of group A infants. The anti-B titres in the first week reached from 80 to 10,240 with a median titre of 640 in 7 specimens obtained from 7 mothers of group B infants. Both these medians are one dilution higher than the corresponding figures in the early postpartum period in our prospective hetero-specific series (Tables 2 and 3). Therefore, our findings tend to agree with those of Reepmaker (15) who stated that the average maternal anti-A titre in cases of ABO hemolytic disease was higher than in normal donors and mothers.

The number of titres available in the late postpartum period in our cases is insufficient to indicate further trends.

2. *Agglutinins in Albumin.* As was pointed out in Part I of this paper it is realized that the actual titres of agglutination in albumin at 37°C are of little quantitative significance in regard to the presence of "immune" agglutinins unless the titre exceeds that of saline agglutination by at least two dilutions, and even then it may only represent a shift in the thermal optimum for saline agglutination. However, this is at least a rough screening test for an immune response in agglutination.

It is interesting to note the number of instances in which there was a two-tube excess of the titre in albumin over that in saline. In this series such an excess was found during the first postpartum week in 6 out of 23 mothers whose infants had disease due to anti-A. On the other hand, it only occurred in 3 out of 32 homospecific O-O and in 4 out of 30 heterospecific O-A pregnancies during the corresponding time period in the prospective series. This finding corresponds to that of Zuelzer and Kaplan (64) who detected a greater spread

between the maternal titres in saline and acacia when affected babies were compared with controls. It indicates that there is indeed a tendency for mothers of affected babies to have an immune type of agglutinin against their infant's A agglutinin.

We have fewer data concerning the maternal titres of anti-B in albumin. Only 8 specimens obtained from 5 mothers of infants suffering from hemolytic disease due to anti-B are available for analysis. However, the trend is evidently the same as with anti-A. In 3 out of 5 patients the titre in albumin at 37°C was at least 2 tubes higher than that in saline at room temperature.

3. *Hemolysins.* Of the 36 group O mothers in this series, 35 were shown to have hemolysins against adult A<sub>1</sub> cells. Only 1 was found to have no hemolysin in a single test performed on the first day after delivery (Case No. 23); this was a gravida 3 with a group A infant of identical Rh type (CDe/cde). Two other group O women with A infants gave negative results for anti-A hemolysin two days after delivery but were shown to have such hemolysins three and eight days later, respectively (Case Nos. 10, 19). The tests in all 36 women were performed within 10 days after delivery with the exception of one mother who was not tested for hemolysins in the puerperium but was known to have had anti-A hemolysin two months earlier (Case No. 24). The 7 women who had affected group B infants are included among the 35 who had *anti-A* hemolysins; they also all had anti-B hemolysin, though its titre in 3 of them was at least one dilution lower than that of the anti-A hemolysin. 21 women with affected A infants

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TABLE 29. *Analysis of three forms of maternal antibody in 34 pregnancies in which all three forms were examined and the infant had ABO hemolytic disease.*

Maternal "immune" antibodies				
Anti-A or Anti-B hemolysin	After neutralization		No. of women in each maternal-fetal blood- group relationship	
	Indirect AHG test	Incomplete agglutinin		
				Total
—	—	—	O-A O-B	0
—	—	+	O-A O-B	0
—	+	—	O-A O-B	1 } 0 }
+	—	—	O-A O-B	6 } 2 }
+	—	+	O-A O-B	1 } 0 }
+	+	—	O-A O-B	6 } 1 }
—	+	+	O-A O-B	0
+	+	+	O-A O-B	14 } 3 }
		Total	O-A O-B	28 } 6 }
				34

also had tests for anti-B hemolysin performed; this was found to be present in 17 (usually at slightly lower titre than anti-A hemolysin) and absent in 4. The anti-A hemolysin titres ranged from 2 to 32; the anti-B titres from 2 to 16.

While it is of interest that nearly all of these mothers had hemolysins against adult  $A_1$  cells in the early postpartum period, it will be remembered that this was also true in our prospective series of heterospecific pregnancies. It agrees with the findings of other workers in cases of ABO hemolytic disease (41) (67). The test for hemolysin against their own infant's cells, recommended by Crawford and his colleagues (41) as being more sensitive,

was performed in 19 mothers in this series. It was positive in 12 of 13 women who had affected group A infants and in 5 out of 6 with affected B infants. The total of 17 positives in 19 cases is not impressively different from the 11 positives in 14 cases obtained in our study of heterospecific pregnancies. The titres in our series were generally somewhat lower than when adult cells were used.

4. *Indirect Antiglobulin Test and Incomplete Agglutinin after Neutralization.* In our series of 36 mothers we obtained 26 positive indirect antiglobulin tests against cells of the infant's group after complete neutralization of saline-agglutinins by A or B group specific substance, and in 1 case

the test was not performed because the agglutinin proved very difficult to neutralize (Case No. 1). In the remaining 9 women the test proved negative; 7 of them had group A and 2 had group B infants. In 8 of these mothers the saline-agglutinin was hard to neutralize and in all 9 cases the infant's serum contained an incompatible sensitizing antibody which must have been derived from the mother. Therefore, it may be presumed that the mother's serum contained a sensitizing antibody which was absorbed in the process of neutralization. In other words, the negative maternal indirect antiglobulin test in these cases represents a "serological failure".

Demonstration of residual agglutinin against cells of the infant's group in an albumin medium after neutralization of the saline-agglutinins was successful in 19 of these 36 mothers, and in 1 case the test was again not performed because of the difficulty in neutralization. In the remaining 16 women the test was negative, 13 being O-A and 3 O-B cases; it is interesting that the indirect antiglobulin test was positive in 8 of these and was also negative in the remaining 8. Conversely, among the 9 cases with a negative indirect antiglobulin test, there was only 1 in whom residual incomplete agglutinin could be demonstrated. So the indirect antiglobulin test was more often positive in our hands. In 7 of the 16 cases with a negative test for maternal incomplete agglutinin after neutralization, there was incompatible agglutinin demonstrable in albumin in the baby's serum, so evidently this antibody is also liable to be masked by the process of neutralization.

The findings in regard to maternal im-

mune antibodies are summarized in Table 29. This shows that in 34 women tests for hemolysin, residual incomplete agglutinin and sensitizing antibody after neutralization were performed simultaneously in the early postpartum period; 28 of these had group A and 6 group B infants. Exactly half (14 with O-A and 3 with O-B relationships) were demonstrated to have all the three forms of immune antibody. Eight of the 34 were shown to have only hemolysin and 7 had both hemolysin and a positive indirect antiglobulin test after neutralization. One case had only a positive antiglobulin test and 1 other had the other two forms of antibody demonstrated with a negative antiglobulin test. No case was without some form of "immune" antibody.

When one compares these findings with those after delivery in our series of heterospecific pregnancies (Table 10) one does not encounter a marked difference. However, the indirect antiglobulin test, and to a lesser extent the demonstration of residual incomplete agglutinin, after neutralization appear positive somewhat more often in the maternal serum when the infant has frank ABO hemolytic disease.

### III. Serological findings in the infants

1. *Indirect Antiglobulin Test.* The indirect antiglobulin test against adult cells of the infant's own group was positive in the sera of all 37 babies. This is largely explained by the criteria of selection as a positive direct or indirect antiglobulin test in the infant was required for inclusion in the series, and the latter is found more commonly than the former in ABO disease. As previously mentioned the im-

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TABLE 30. Analysis of direct anti-human globulin (AHG) tests, using commercial and a potent native serum, in 35 infants with ABO hemolytic disease.

	No. of cases tested	Test positive	Test negative
Comm. AHG Serum	35	13	22
Dilutions of Potent Native AHG Serum			
1:10	27	1	26
1:50	26	6	20
1:100	27	24	3
1:200	24	22	2
1:500	23	15	8

versal finding of sensitizing antibody in these infants would indicate their presence in the mother, too, but they were only demonstrable in 26 out of 35 maternal sera after complete neutralization.

2. *Incompatible Agglutinin in Albumin.* The presence of agglutinin against red cells of the infant's group was demonstrated in an albumin medium in 15 out of 21 babies' sera. This included 12 out of 18 group A and 3 out of 3 group B infants. The results suggest that the test is less constantly positive than the indirect antiglobulin test in affected infants. However, it is admitted that a bias may have

been introduced by our method of selecting cases for analysis. In any case, as pointed out in Part II, the test is also technically less satisfactory.

3. *Direct Antiglobulin Test.* In Part II the advantages of using native absorbed antiglobulin serum in various dilutions for the detection of red cell sensitization by ABO antibodies were demonstrated, and this method was shown to be more sensitive than the direct antiglobulin test performed with commercial serum. It was found that the optimal dilution of native antiglobulin serum for this purpose was 1:100, with 1:200 as second best (see Part II, Table 16). In the 37 infants with ABO hemolytic disease, similar results were obtained. The direct antiglobulin test, as performed with commercial serum most suitable for the detection of Rh antibodies, was only positive in 13 out of 35 cases tested (Table 30). On the other hand, the test as performed with titrated native absorbed antiglobulin serum proved positive in 24 of 27 cases at a dilution of 1:100 and in 22 of 24 cases at a dilution of 1:200. When both methods were used the test with commercial serum was never positive alone.

TABLE 31. Comparison of results of direct anti-human globulin (AHG) tests performed by two different techniques in 26 infants with ABO hemolytic disease as related to time after birth.

	Within 12 hours after birth		13-24 hours		25-48 hours		49-72 hours		Over 72 hours	
	Comm. AHG serum	Native AHG serum Dil. 1:100	Comm. AHG serum	Native AHG serum Dil. 1:100	Comm. AHG serum	Native AHG serum Dil. 1:100	Comm. AHG serum	Native AHG serum Dil. 1:100	Comm. AHG serum	Native AHG serum Dil. 1:100
Positive	10	14	4	7	5	13	3	4	0	3
Negative	5	1	3	0	10	2	4	3	9	6
Total tests per time period	15		7		15		7		9	

Direct antiglobulin tests were performed by both methods in 26 infants with ABO disease, often repeatedly. If these are analyzed according to the time period after birth the results are as shown in Table 31. This demonstrates the superiority of the titration method at all periods. It also suggests that the direct antiglobulin test performed with commercial serum, if initially positive, tends to become negative sooner than when the titration method is used with concentrated serum. Very roughly, the test with commercial serum was *likely* to prove negative after the first day, the test with diluted native absorbed serum only after the third day.

### Discussion

The clinical picture in the 37 infants described here is in keeping with that in other reported cases of hemolytic disease due to ABO incompatibility. The proportion of first-born infants (9 out of 37) is slightly lower than in other reported series, where it usually amounts to between one-third and one-half of the total number of cases. It is interesting that approximately one-third of the mothers who had previous offspring gave a history of marked neonatal jaundice in older siblings of our patients, though it is known that the disease is less regularly progressive in successive children than that due to Rh incompatibility. The main clinical manifestation of the disease was jaundice which had usually become manifest within 24 hours of birth. Its severity was the main factor in prognosis and determined the necessity for the therapeutic use of replacement transfusion. Anemia represented a lesser phenomenon; it was only noted clinically in 5

and was demonstrable by abnormally low hemoglobin in 10 out of 35 infants and was never severe in the first week. Reticulocytosis occurred in over half of the affected infants and an increase in the nucleated red cell count was also present in a somewhat lower proportion of the patients. Spherocytosis of some degree was found universally when it was looked for, whereas the red cell fragility test in our hands proved less commonly abnormal.

No fatalities or instances of kernicterus occurred in this series of 37 infants, and this may be largely due to the ready use of replacement transfusions. These were undertaken in no fewer than 12 of the 37 infants and were repeated in 3 of them. In this connection it is of interest to recall that the mortality rate in Reepmaker's (15) series of 241 patients with ABO hemolytic disease was 10.8% and that 4 out of 38 patients with this condition reported by Zuelzer and Kaplan (64) developed kernicterus. In the present state of our knowledge it would seem reasonable to perform a replacement transfusion whenever the serum bilirubin level, and particularly the indirect component, approaches the level of 20 mg %, as is usually advised in erythroblastosis due to Rh iso-immunization. Levine (26) has also suggested that replacement transfusion is indicated when the reticulocyte count exceeds 20%; we had two cases in whom this was known to occur, and one of these received an exchange transfusion.

In regard to the serological findings we were particularly interested to compare this series of infants exhibiting frank hemolytic disease with those whom we had followed prospectively in homosppecific and heterosppecific pregnancy. The mor-

nal anti-A titres in saline in the early postpartum period did not differ markedly in cases of hemolytic disease as compared to ordinary heterospecific pregnancy. Although isolated strikingly high titres were observed in the mothers of affected infants, the median of their saline titres was only one dilution higher than in the heterospecific series. It was notable that among the mothers with diseased babies there was a slightly higher proportion whose agglutinin titre in albumin exceeded that in saline by at least two tubes, as compared to the prospective series. This indicated the "spread" between the maternal anti-A titres in saline at room temperature and in colloid at body temperature which appears to be a feature of the immune response and was previously found by Zuelzer and Kaplan (64) in a series of affected babies.

Hemolysins against adult A<sub>1</sub> cells were almost universally present in the group O mothers of these affected children. Hemolysins which were effective against the less reactive cells of their own infants were found in 17 out of 19 mothers. Unfortunately, the demonstration of hemolysins against either type of red cell is of little diagnostic value as we found a similarly high proportion of positive results in women with ordinary heterospecific pregnancies (see Part I). Crawford and his colleagues (41) hoped that the test performed against the infant's cells would prove more specific for hemolytic disease in the child, but as noted previously, we obtained 11 positive tests of this kind in 14 women during heterospecific pregnancies, and on the other hand Zuelzer and Kaplan (64) only demonstrated a maternal hemolysin against the red cells

of newborn A<sub>1</sub> infants in 13 out of 18 cases of hemolytic disease due to anti-A.

At present the most significant procedure in regard to the maternal serum would appear to be the indirect antiglobulin test against A<sub>1</sub> or B cells after neutralization of saline-agglutinin. As was shown earlier in this paper, positive tests of this kind indicate that the infant in a heterospecific pregnancy has approximately a 50% chance of exhibiting a positive direct antiglobulin test and about a 25% chance of having frank hemolytic disease. In the present series of 36 mothers with affected infants a positive indirect antiglobulin test against cells of the infant's group after neutralization was obtained 26 times (with one further inconclusive result), and the remaining cases presumably represented "serological failures" in which the sensitizing antibody was masked during neutralization. The demonstration of a residual agglutinin acting against cells of the infant's group in an albumin medium after neutralization was only successful in 19 of these 36 mothers and thus proved somewhat less useful as a diagnostic aid. Both tests appeared positive somewhat more often in the maternal serum when the infant has frank ABO disease than in ordinary heterospecific pregnancy.

In regard to serological findings in the infant, it is not surprising that the indirect antiglobulin test given by the serum against adult cells of the baby's group was positive in every case, as this was largely a criterion for admission into the series. The alternative mode of demonstrating an incompatible antibody in the infant's serum by means of agglutination of homologous cells in an albumin medium was only successful in 15 out of the 21

children and was technically less satisfactory. It may be that the use of activated papain represents a better technique for the detection of incomplete agglutinins in the infant (70) (53).

In view of frequent statements in the literature that the direct antiglobulin test is rarely positive in cases of hemolytic disease due to ABO incompatibility, it was interesting to see the high proportion of positive results obtainable by the use of native absorbed antiglobulin serum in serial dilution. This might be partly ascribed to our method of selecting these cases, but they were accepted into the series even if only the indirect antiglobulin test was positive and the superiority of the titration method over the use of commercial antiglobulin serum is clearly demonstrated. This relates not only to the larger number of positive results but also to the longer period after birth during which such a result may be obtained. Naturally, as the direct antiglobulin test is made more sensitive by this modification it will show up more cases of only mild hemolytic disease, as was illustrated in Part III. Nonetheless, it should prove a most useful diagnostic tool in selecting for the clinician those infants born of heterospecific pregnancy who are liable to develop frank hemolytic disease.

### Summary

A detailed analysis of 37 cases of hemolytic disease due to ABO incompatibility is presented, with particular reference to the serological findings. These 37 infants were the offspring of 36 group O mothers, and all satisfied the criteria for ABO hemolytic disease as laid down in a

previous section of this paper. Thirty of them belonged to group A and 7 to group B. Nine were first-born children. The clinical manifestations corresponded closely to those described in other series. The main presenting symptom was early jaundice, and its severity largely determined the prognosis and treatment. Significant anemia was also present in 10 of the infants, but was usually mild. The hematological findings are discussed: the almost universal presence of spherocytosis, the occurrence of reticulocytosis in over half of the cases tested and the somewhat less frequent finding of a raised nucleated red cell count are described. The changes in serum bilirubin levels are also outlined.

Twelve of the 37 infants received replacement transfusions (repeated in three instances), and one further child received two simple transfusions. No fatalities and no cases of kernicterus occurred.

The median maternal saline-agglutinin titre during the first week after delivery in this series was one dilution higher than that encountered in our prospective study of heterospecific pregnancies. Further, in a slightly higher proportion than in the latter group, the maternal anti-A agglutinin titre in albumin at 37°C exceeded that in saline at room temperature by at least two dilutions. This is taken to indicate a form of immune response in agglutination.

Hemolysins against adult A<sub>1</sub> cells were demonstrated in 35 out of 36 maternal sera, but were of little diagnostic aid as they were equally common in our ordinary heterospecific series. Hemolysins against infants' A cells were demonstrated in 17 out of 19 maternal sera, but proved hardly more helpful. After neutralization of saline-

agglutinin by A or B group specific substance the maternal sera gave a positive indirect antiglobulin test in 26 out of 36 cases and residual agglutination of such cells in albumin in 19 out of 36 cases. The former test appeared practically more useful. Both reactions were somewhat more often positive in these mothers of affected infants than in the ordinary heterospecific pregnancies studied previously.

Partly because of the manner in which they had been selected, the infants always gave a positive indirect antiglobulin test against adult cells of their own group. The presence of an incompatible agglutinin acting in an albumin medium was also demonstrated in 15 out of 21 infants' sera.

This test appeared less useful and technically satisfactory than the indirect antiglobulin procedure.

The direct antiglobulin test performed with commercial antiglobulin serum was positive in 13 of the 35 infants tested, whereas the test performed with titrated native absorbed antiglobulin serum proved positive in 24 of 27 cases at a dilution of 1:100. It was also shown that the result was likely to be negative after the first day when commercial serum was used and only after the third day with the titration method.

These results are discussed in relation to the findings of other workers.

## Epilogue

In the work reported here we have undertaken a prospective study of the effects of ABO incompatibility on mother and infant during and after pregnancy. We have compared the course of homo-specific and heterospecific pregnancies, with particular emphasis on serological changes, and we have analyzed the incidence and special features of the "hemolytic disease" which sometimes results from the incompatibility in heterospecific cases. While many points of interest emerge from our study, the reader should be warned that the elucidation of basic differences in regard to clinical, hematological, biochemical and serological data in homospecific versus heterospecific pregnancy, and in uneventful heterospecific pregnancy versus such pregnancy with disease in the newborn, necessitates an analysis of very large numbers of cases if the differences are to assume statistical significance. Hence, in many instances, we have only been able to indicate trends rather than statistically proven facts. This difficulty applies particularly to titre changes, as these are notoriously difficult to analyze statistically.

It remains largely obscure why some group O mothers develop more anti-A or anti-B antibodies than other women with an identical blood group relationship to the infant, and it is poorly understood

why some babies are much more severely affected by the maternal antibodies than others with equal maternal titres. Many hypotheses have been formulated to answer these questions, but we still lack understanding of some of the basic processes underlying ABO hemolytic disease.

In conclusion, it may be fitting to point out a few outstanding problems:

1. The chemical and immunochemical analysis of blood group substances (71) (72) and the immunological differentiation of A and B subgroups (73) and of the anti-A and anti-B antibodies in their various forms (74) (75) are being actively pursued in various centres. This should lead to the development of more accurate diagnostic tests in the disease and perhaps also to better methods of prevention and treatment.

2. It is now generally agreed that the disease occurs predominantly in children born to group O mothers. This presumably indicates that such women are liable to produce a particularly potent antibody in the ABO system which does not occur in group A or B persons. Wiener and his colleagues (76) (77) (78) have suggested that this is an antibody against a factor C which is present in group A, B or AB persons and that this particular antibody can therefore only be found in group O women and may be the main cause of

hemolytic disease in their offspring. Alternatively, it has been considered that the harmful antibody in group O women may be a "linked" cross-reacting anti-A and anti-B (79) (80). A recent study by Zuelzer and his colleagues (81) in a relevant twin pregnancy, however, supports the view that maternal group O sera contain multiple fractions of anti-A and anti-B antibodies with different partial specificities, which together determine the degree of cross-reaction, rather than a single "linked" antibody. Clearly this matter deserves further study.

3. The growing awareness of occasional breaks in the placental barrier, particularly at the time of delivery (82) (83) is likely to impinge on the field of hemolytic disease. While fetal-maternal transfusions are known to occur in late pregnancy and are liable to lead to severe anemia in the infant, it is also conceivable that maternal-fetal transfusion at the time of delivery may precipitate hemolytic disease. In heterospecific pregnancy such a transfusion of group O blood from the mother could be dangerous if it contained immune anti-A or anti-B to a high titre. These antibodies could cause ABO hemolytic disease in the infant (which is closely similar to a transfusion reaction), and the resulting anemia might well be partly masked by the group O blood received from the mother. The further investigation of such placental breaks will therefore be of much interest, and the resulting changes in the types and amounts of hemoglobin, in the levels of bilirubin and in the morphology and group of the red cells in mother and infant are a fruitful field for research.

4. Recently, the phenomenon of im-

munological tolerance has attracted increasing attention. It has been demonstrated that animals exposed to foreign cells or substances during fetal development or in the early neonatal period will often fail to make antibodies against this particular antigen in later life (84) (85). This phenomenon has a bearing on hemolytic disease. For instance, a group O woman whose mother belonged to group A and was a secretor, is likely to have been exposed to A antigen in fetal life and might thus be expected to have a reduced capacity for making anti-A later. She might therefore be less liable to be responsible for hemolytic disease in a group A offspring than another group O woman whose mother belonged to group O (62). To our knowledge, this matter has not yet been investigated in ABO incompatibility. Studies in regard to Rh immunization so far suggest that mothers exposed to the antigen in fetal life are less likely to become sensitized later (86), but do not show a significant reduction in the incidence of hemolytic disease among their offspring (87) (88). The possibility of causing immunological tolerance artificially in later life or of desensitizing those mothers who have dangerous antibodies still remains remote.

5. There have been recent advances in the understanding of bilirubin metabolism in the newborn. It is now evident that bilirubin is conjugated with glucuronic acid in the liver prior to its excretion, and that there may be much variation in the maturation of the enzyme system involved in this conjugation (89) (90) (91) (92). The study of these processes may lead to a more successful management of hemolytic disease (93) (94).

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